JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. III WASHINGTON, D. C., DECEMBER 15, 1914 No. 3

CHANGES IN COMPOSITION OF PEEL AND PULP OF RIPENING BANANAS

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INTRODUCTION

In attempting to determine the effect of temperature on the rate of respiration of the banana (*Musa sapientum*), it was found that the rate of respiration increased very rapidly during ripening(13)[1], as, indeed, had been observed by Gerber (12), and the bananas maintained themselves during ripening at temperatures distinctly above that of their surroundings, in consequence of the intensely active respiration. The phenomenon of self-heating suggested the need of experiments in a calorimeter to determine the amount of heat evolved in relation to carbon dioxid formed and oxygen consumed. The necessity of accompanying such studies with analyses of samples of the bananas under observation led to the analytical work recorded in this paper.

The chemical changes which occur in the banana during ripening have been studied by Buignet (5); Corenwinder (8, 9, 10), Marcano and Muntz (17); Ricciardi (20); Doherty (11); Colby (7); Gerber (12); Balland (4); Atwater and Bryant (1); Chace, Tolman, and Munson (6); Bailey (2, 3); Prinsen Geerligs (18); Tallarico (21); Jähkel (14); Yoshimura (22); and Reich (19). The results have invariably been expressed in terms of the percentage of the pulp of the fruit when analyzed, and the data are, in consequence, on a constantly shifting basis, as the peel continuously loses weight and the weight of the pulp steadily increases. An accurate account of the chemical changes for use in exact biochemical studies was therefore lacking.

Four ripening experiments were made. In two experiments bunches of green bananas were ripened in a large respiration calorimeter designed for experiments with man. In the third and fourth experiments studies were made, in a specially designed ripening chamber, of the uniformity

[1] Reference is made by number to "Literature cited," pp. 202–203.

with which ripening occurs in different bunches of bananas and of the rate of starch hydrolysis during ripening in relation to changes in the rate of respiration. The first and second experiments were made in cooperation with Messrs. C. F. Langworthy and R. D. Milner, Nutrition Investigations, Office of Experiment Stations, who measured the weight changes on ripening, the carbon dioxid, the water and the heat evolved, and the oxygen absorbed. The composition was determined before and after ripening. Green bananas selected from commercial shipments just received from the West Indies were used in all cases.

FIRST EXPERIMENT

Six bunches of green bananas were used. They were evenly matured, so far as could be judged from the comparative intensity of the green color. The top and the bottom of the stem of each bunch were trimmed smoothly, and all injured fruits whose pulp might have been infected as a result of mechanical injury were removed.

For analysis, six fruits were cut from each of five bunches and eight fruits taken from the sixth bunch, which was larger than the others. The samples were taken from the inner and the outer portions of the "hands" at the top, the middle, and the bottom of each bunch, cutting them off where the short stem by which the bananas are attached to the stalks was most constricted. After ripening, samples were removed from the same "hands" from which samples had been previously taken, but they were cut from places removed from where the preceding samples had been attached, in order to avoid possible effects of local stimulus to adjacent fruits due to cutting. No indication of such effect, however, was noticeable.

The samples of green or ripe bananas were first weighed and then separated into peel and pulp. The peels of green bananas adhere closely to the pulp, and appreciable losses from evaporation are unavoidable. After separation, the peel and the pulp were weighed, to determine their respective proportions and the losses from evaporation. To express the analyses in terms of the original bananas, it was necessary to correct for this evaporation. To this end the loss in weight on peeling was arbitrarily divided equally between the respective weights of peel and pulp, and the percentages of peel and pulp were then calculated. The samples of peel and pulp were ground by passing them through a food chopper.

The methods of analysis used in this and succeeding studies were as follows:

SOLIDS were determined by evaporation in vacuo at 70° C.

ASH AND SOLUBLE ALKALINITY OF ASH were determined by the official methods.[1]

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), p. 38, 102. 1908.

NITROGEN determinations were made by the official methods[1] by Mr. T. C. Trescot, of the Bureau of Chemistry.

ETHER EXTRACT was determined by exhausting with alcohol, and determining the ether extract in residue and alcohol-soluble extract. In case of the alcohol-insoluble extract the alcohol was evaporated, the residue transferred to a separatory funnel by use of water and ether, and extracted repeatedly with ether. These extracts were then combined, washed with water, and evaporated in tared flasks.

CARBOHYDRATES were determined by treating weighed samples with 80 per cent alcohol in Soxhlet extractors, using several portions of alcohol so as to avoid giving the larger proportion of the sugars a prolonged heat treatment. The residues were dried and weighed, and the weighed portions used in the estimation of starch and pentosans. The extracts were mixed with a little calcium carbonate, evaporated on the steam bath in a current of air, avoiding evaporation to dryness, and then taken up in water, treated with lead acetate, and made up to a known volume. After filtering, the excess of lead was removed by use of dry sodium oxalate.

REDUCING SUGAR AND SUCROSE were determined in this solution by applying the copper-reduction method of Munson and Walker,[2] before and after inversion.[3]

STARCH. To determine starch, 3-gram samples of the alcohol-insoluble material were transferred to 300 c. c. flasks, hydrolyzed with hydrochloric acid as directed in the official method,[4] and the dextrose determined by the copper-reduction method of Munson and Walker.[5]

PENTOSANS were determined in the alcohol-insoluble residues by the provisional method of the Association of Official Agricultural Chemists.[6]

Although at the time it was supposed that due precautions had been taken to prevent inversion of sucrose, unfortunately it is not improbable that more or less inversion may have occurred, owing to the failure to heat the alcohol extract to boiling.

When removed, the bananas were of a bright clear yellow, with a little green showing at the tips. They were entirely free from decay. The fruit surfaces were moist and waxy, indicating that the humidity had been high. During ripening, the calorimeter record showed that the temperatures had varied from 18.8° to 20.4° C., average 20.1° C. The oxygen content of the air supplied varied from 20.4 to 10.8 per cent by volume, average 15.5 per cent, probably sufficient for normal ripening.

The bananas weighed 137.71 kg. when placed in the calorimeter and 132.37 kg. when withdrawn, a loss in weight of 3.88 per cent. The

[1] Op. cit., p. 7.
[2] Op. cit., p. 41.
[3] Op. cit., p. 241
[4] Op. cit., p. 241.
[5] Op. cit., p. 53
[6] Op. cit., p. 54.

fruit consisted of 41.72 per cent of peel and 58.28 per cent of pulp when green and of 37.85 per cent of peel and 62.15 per cent of pulp when ripe. Calculated on the basis of the original bananas, the percentage of ripe peel was 36.38, a loss of 5.34 per cent of its weight. On the same basis the percentage of pulp was 59.73, a gain in weight of 1.45 per cent. Expressed in terms of the original bananas, the solids in the peel decreased from 5.21 to 4.88 per cent, the water from 36.51 to 31.50 per cent, about two-thirds of the starch passed into sugars, and the pentosans decreased slightly. The ash, the alkalinity of ash, the nitrogen, and the ether extract did not change materially. There was an apparent net loss in carbohydrates of 0.46 per cent.

Expressed in the same way, the solids in the pulp decreased from 16.93 to 16.74 per cent. The water increased from 41.35 to 42.99 per cent. The starch content changed from 13.15 to 2.40 per cent, the reducing sugars from 0.37 to 10.34 per cent, and the sucrose from 0.48 to 1.52 per cent. There was a net loss in carbohydrates of 0.88 per cent, due to respiration. The pentosans changed from 0.40 to 0.18 per cent. As in the peel, the ash, the alkalinity of ash, the nitrogen, and the ether extract in the pulp did not undergo marked changes in the amounts present. See Table I.

TABLE I.—*Composition of bananas before and after ripening in respiration calorimeter*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF PEEL OR PULP WHEN ANALYZED

Part of fruit.	Percentage of whole fruit.	Composition.										
		Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein, N × 6.25.	Ether extract.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.	Pentosans.
Peel:												
Green bananas	41.72	87.52	12.48	1.32	0.82	0.61	0.89	0.70	0.23	4.84	8.98	0.91
Ripe bananas	37.85	86.59	13.41	1.54	1.04	.64	1.08	3.71	.10	1.94	6.24	.69
Pulp:												
Green bananas	58.28	70.97	29.03	.87	.49	1.20	.24	.64	.83	22.56	26.28	.69
Ripe bananas	62.15	71.98	28.02	.85	.47	1.24	.24	17.31	2.54	4.02	6.36	.30

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS[a]

Peel.												
Green bananas	41.72	36.51	5.21	0.55	0.34	0.26	0.37	0.29	0.10	2.02	3.75	0.38
Ripe bananas	37.85	31.50	4.88	.58	.38	.23	.39	1.35	.04	.71	2.27	.32
Pulp:												
Green bananas	58.28	41.35	16.93	.51	.29	.70	.14	.37	.48	13.15	15.32	.40
Ripe bananas	59.73	42.99	16.74	.51	.28	.74	.14	10.34	1.52	2.40	3.80	.18

[a] Percentage of loss in weight on ripening, 3.86.

SECOND EXPERIMENT

In the second experiment similar composition changes occurred. In addition, it was demonstrated that the banana stem does not contribute materially to the fruit during ripening. The details of this experiment are as follows:

Four bunches of green bananas, consisting of the most evenly ripened fruit, judging by color, of a lot of eight bunches of the green fruit, were placed in the calorimeter after sampling and weighing. Six fruits had been removed as samples from each of two bunches of green bananas and eight from each of the others.

During ripening the temperature varied from 20.9° to 24.2° C., averaging 23.1° C.; the oxygen content of the air varied between 20.0 and 6.1 per cent by volume, averaging 14.1 per cent; and the humidity remained high, as before. When removed, all of the fruits were thoroughly ripe, and indeed one bunch was slightly overripe. Several specimens on this bunch were beginning to spoil. The skins were bright golden yellow, and the fruit surfaces were waxy and moist. No browning occurred, except where the bananas were superficially injured.

After weighing, the bunches were again sampled. In the analyses determinations of protein, ash, and ether extract were omitted, as the quantities of these substances present had been found to change but very slightly during ripening.

The four bunches remaining after selection of the bananas for the respiration calorimeter were used in the study of the composition of the green stem. Mr. W. H. Evans, of the Office of Experiment Stations, suggested that possibly the stem contained reserve materials supplied to the fruit during ripening. The bananas were detached from the stems in the same manner as when taking samples for analysis, leaving on the stems the stubs of the short stems by which the bananas were attached. The percentage of stems was 5.04 per cent. They were finely divided in a shredding machine and analyzed, using the methods employed for the analysis of peel and pulp. At the conclusion of the ripening experiment in the calorimeter the weight of the stems of the ripened bananas was determined and the stems then ground and analyzed.

The original weight of the four bunches of green bananas placed in the calorimeter after sampling was 75.90 kg. They suffered a loss upon ripening of 6.36 kg., or 8.38 per cent.

The bananas after ripening consisted of 95.30 per cent of fruit and 4.70 per cent of stem. Assuming that the fruit and stalks lost weight in equal proportions, the respective weights of green fruits and stems when placed in the calorimeter were 72.33 and 3.57 kg. The proportions of pulp and peel of the green bananas were 58.22 and 41.78 per cent, respectively. The entire bunches of green bananas as placed in the calorimeter after sampling consisted therefore of 55.48 per cent of

pulp, 39.82 per cent of peel, and 4.70 per cent of stem. When ripe, the bunches consisted of 63 per cent of pulp, 32.30 per cent of peel, and 4.70 per cent of stem. Based, however, upon the weight of the original bunches of green bananas, the proportion in ripe bananas was 57.72 per cent of pulp, 29.59 per cent of peel, and 4.32 per cent of stem, a total of 91.63 per cent.

The analytical data are given in Table II in terms of percentage of pulp and peel as analyzed (corrected for loss in weight in peeling), in terms of the original whole bananas, and in terms of the entire bunch of bananas—i. e., including the stem. The ripening period was longer than in the first experiment in the calorimeter, more starch disappeared, and more sugars formed, while the gains of water in pulp and losses in peel were greater. The study of the composition of the stem before and after ripening showed that the changes which occur in it during the ripening process are so slight as to be insignificant. The percentage of loss in solids, on the basis of the original bananas, was but 0.04 per cent.

TABLE II.—*Composition of bananas before and after ripening in respiration calorimeter*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF PEEL, PULP, AND STEM OF THE BANANAS BEFORE AND AFTER RIPENING

Part of fruit.	Percentage of whole fruit.	Composition						
		Water.	Solids.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.	Pentosans.
Peel:								
Green bananas	41.78	88.28	11.72	0.76	0.02	4.43	8.55	0.83
Ripe bananas	33.89	85.12	14.88	4.42	.12	1.05	6.77	.96
Pulp:								
Green bananas	58.22	70.76	29.24	.71	.02	24.10	27.03	.68
Ripe bananas	66.11	73.00	27.00	13.81	8.50	1.17	3.44	.35
Stem:								
Green bananas	[a] 4.70	90.19	9.81	.63	.17	1.59	7.02	.....
Ripe bananas	4.70	88.97	11.03	.30	.04	1.83	8.72	.....
COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS [b]								
Peel:								
Green bananas	41.78	36.88	4.90	0.32	0.01	1.85	3.57	0.35
Ripe bananas	31.05	26.43	4.62	1.37	.04	.51	2.10	.33
Pulp:								
Green bananas	58.22	41.20	17.02	.41	.01	14.03	15.74	.40
Ripe bananas	60.57	44.22	16.35	8.37	5.15	.71	2.07	.21
COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE STEM OF GREEN BANANAS [b]								
Peel:								
Green bananas	39.82	35.15	4.67	0.30	0.008	1.76	3.40	.. ..
Ripe bananas	29.59	25.19	4.40	1.31	.04	.49	2.00	.. ..
Pulp:								
Green bananas	55.48	39.26	16.22	.39	.01	13.37	15.00	....
Ripe bananas	57.72	42.14	15.58	7.97	4.91	.68	1.97	.. ..
Stem:								
Green bananas	4.70	4.18	.52	.03	.01	.08	.33	.. .
Ripe bananas	4.32	3.84	.48	.01	.002	.08	.38	.... .

[a] It is here assumed that the same proportion of stem is present in the green as in the ripe bananas.
[b] Percentage of loss in weight on ripening, 8.38.

THIRD EXPERIMENT

It now seemed well to observe the composition of the banana of commerce repeatedly at several stages during its ripening, in order to determine the uniformity of the changes which occur in bananas from different bunches. Four bunches of bananas were ripened in a specially constructed humidity chamber and each bunch was sampled three times during its ripening. This experiment demonstrated that the changes in ripening in the four bunches were remarkably uniform, while the data secured in the first two experiments were repeatedly confirmed. In addition, the study gives a good idea of the composition of bananas when just ripe enough to be edible and also when very ripe.

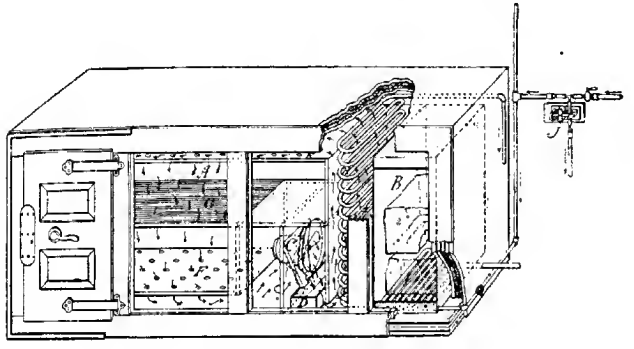

FIG. 1.—Constant-temperature humidor.

The detailed account of the experiment is as follows:

The humidity chamber (fig. 1) consisted of a large case, of cold-storage construction, divided by an insulated wall into two compartments, A and B. Compartment A was so arranged that it could be kept at constant temperature and humidity at temperatures below that of the room. Ice was placed in compartment B. In A the air was circulated continuously by an electric fan. The course of the air current was as follows: From the fan it was deflected by the baffle C to play upon the surface of water in the tank D. The water was here kept at constant level by a device not shown and was warmed by an electrically heated immersion heating coil, also not shown. The air then passed up through the vertical cooling coil H. The air, moisture laden and cooled, was then delivered through holes in the false ceiling E to the rest of the compartment, whence it was drawn back to the fan through holes in the false bottom F. Detached bananas or other objects were placed on the false bottom F or on the slatted shelf G. When bunches of bananas were being ripened, shelf G was removed and the fruit was suspended from hooks in the false ceiling.

Compartment B was lined with galvanized iron. At the bottom the horizontal coil I was connected through the partition with the vertical coil H in compartment A. The ice rested directly upon coil I. To cool compartment A, tap water was supplied to coil I and the ice-cold water displaced in coil I passed into coil H. Automatic delivery of cold water to coil H was accomplished by the operation of the sounder J, which in turn was controlled by the thermostat (not shown) in compartment A. The form of the thermostat has already been illustrated (13). The low-tension circuit opened and closed by the thermostat operated a relay in which a 110-volt D. C. circuit was opened and closed. In the 110-volt D. C. circuit a 32-candlepower carbon-filament lamp and the sounder J were placed in series. When the circuit was closed, the lever of the sounder compressed a rubber tube through which a slow stream of tap water was passing to waste. The water was then delivered to coil I. The sounder was of a resistance of 4 ohms.

The constancy of the apparatus is from 0.1° to 0.2° C., depending upon a number of factors, of which the external temperature and the rate of water flow are perhaps the most important. The humidity remained high and constant. The apparatus is capable of operating continuously for weeks at a time in the neighborhood of 20° C. in hot weather, with little or no attention, except that of being supplied with ice. The cold-storage features of the humidity chamber, without which it would be of little value for the purpose of operating at temperatures below that of the room, were suggested by Mr. S. J. Dennis, of the Bureau of Plant Industry.

Four large bunches of average-sized green bananas, carefully trimmed and sampled, were weighed and suspended in the humidity chamber and the bananas allowed to ripen. When their color had just changed from green to yellow, the tips of the fruit being still green, each lot was weighed, sampled as before, again weighed, and allowed to ripen further. The temperature was kept at 20° C. during ripening, and the air in the humidor was constantly renewed. When the bunches had become very ripe, most of the fruits showing slight superficial browning, the bunches were weighed and samples again taken. The pulp of the bananas was now soft, tender, and somewhat mealy. It was entirely free from translucent semiliquid portions and from decay. On weighing the bunches at this time many fruits broke off, and the experiment was discontinued.

The records of the weights permit reference of the results to the basis of the original bunches, assuming, as in the previously described studies, that the stems lost weight at the same rate as the bananas themselves. The analytical data are shown in Table III. In all cases nearly all of the starch of the peel and the pulp gradually passed into sugar. For completeness the ash, the alkalinity of the ash, and the protein were determined in both pulp and peel. As in the previous study, the changes

in the quantities of these ingredients present were insignificant. In the peel the transformation of starch into soluble carbohydrates was more rapid during the change from green to yellow than subsequently. In the pulp also the transformation of starch was the most rapid during the change in color of the bananas from green to yellow. By the end of this period about two-thirds of the starch had passed into soluble carbohydrates. During the subsequent ripening in each case a large proportion of the remaining starch in the pulp became converted into sugars. Some variation appears in the amounts of sucrose and invert sugar formed on ripening in the pulp of the bananas from the several bunches, but unfortunately a defect in the method existed (see p. 189), and more or less inversion of sucrose may have occurred during analysis. The figures for sucrose are probably slightly high and those for reducing sugars correspondingly low. Striking features of each set of analyses are the water changes. The peels lost water at uniform rates; the pulps all gained water—most rapidly after turning yellow.

TABLE III.—*Composition of bananas during ripening on the stem in the humidity chamber*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGES OF PEEL AND PULP OF THE BANANAS BEFORE AND AFTER RIPENING

Serial No.	Part of fruit.	Interval of keeping in humidity chamber.	Loss in weight on ripening.	Percentage of whole fruit.	Composition of peel or pulp.								
					Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.
	Peel:	*Days.*											
	Green bananas	0	0.00	40.30	89.06	10.94	1.45	0.95	0.64	0.90	0.23	4.50	8.31
	Ripe bananas	6	4.46	36.76	87.64	12.36	1.71	1.10	.68	3.26	.06	2.00	6.86
1065	Full ripe bananas	13	10.04	31.15	84.31	15.69	2.09	1.38	.87	3.82	.34	1.98	8.30
	Pulp:												
	Green bananas	0	0.00	59.70	72.04	27.06	.90	.53	1.04	.27	.37	21.54	24.88
	Ripe bananas	6	4.46	63.24	72.94	27.06	.94	.52	.97	16.41	1.02	5.42	7.92
	Full ripe bananas	13	10.04	68.85	75.98	24.02	.86	.49	.93	13.56	5.35	.76	2.89
	Peel:												
	Green bananas	0	0.00	40.15	87.75	12.25	1.37	.86	.92	.69	.21	4.94	9.49
	Ripe bananas	5	3.33	37.60	87.09	12.91	1.55	.95	.88	3.14	.51	2.54	7.52
1066	Full ripe bananas	13	9.43	31.22	83.59	16.41	1.91	1.20	1.07	4.77	.26	1.94	8.08
	Pulp:												
	Green bananas	0	0.00	59.85	72.21	27.79	.88	.52	1.30	.64	.30	22.55	25.92
	Ripe bananas	5	3.33	62.40	72.76	28.24	.90	.60	1.25	7.69	9.37	6.49	9.00
	Full ripe bananas	13	9.43	68.78	75.70	24.30	.82	.47	1.16	14.55	5.07	.75	2.76
	Peel:												
	Green bananas	0	0.00	40.35	88.13	11.87	1.37	.87	.88	.90	.12	4.42	8.74
	Ripe bananas	5	3.17	37.45	87.32	12.68	1.52	.96	.79	2.81	.65	2.33	7.09
1067	Full ripe bananas	13	8.90	30.80	83.53	16.47	1.96	1.23	1.21	4.50	.19	1.98	8.33
	Pulp:												
	Green bananas	0	0.00	59.65	72.12	27.88	.92	.54	1.37	.41	.51	22.43	26.05
	Ripe bananas	5	3.17	62.55	71.87	28.13	.95	.50	1.32	7.36	9.17	6.56	8.95
	Full ripe bananas	13	8.90	69.20	75.40	24.60	.86	.48	1.23	13.12	6.70	.77	2.78
	Peel:												
	Green bananas	0	0.00	43.44	88.74	11.26	1.32	.86	.73	.90	.15	4.10	8.33
	Ripe bananas	7	3.98	39.70	87.64	12.36	1.51	.98	.74	2.99	.11	2.06	6.62
1068	Full ripe bananas	13	8.02	34.27	85.38	14.62	1.83	1.20	.93	3.56	.16	1.77	7.49
	Pulp:												
	Green bananas	0	0.00	56.56	71.90	28.10	.85	.49	1.20	.29	.43	22.51	26.22
	Ripe bananas	7	3.98	60.33	72.07	27.93	.90	.48	1.14	14.69	1.08	6.88	9.66
	Full ripe bananas	13	8.02	65.73	74.92	25.08	.80	.45	1.06	9.91	6.81	1.01	3.18

TABLE III.—*Composition of bananas during ripening on the stem in the humidity chamber*—Continued

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS

Serial No.	Part of fruit.	Interval of keeping in humidity chamber	Loss in weight on ripening.	Percentage of whole fruit.	Composition of peel or pulp.								
					Water.	Solids.	Ash.	Alkalinity of ash as K_2CO_3.	Protein.	Reducing sugar as invert.	Sucrose.	Starch.	Alcohol-insoluble solids.
	Peel:	*Days.*											
	Green bananas....	0	0.00	40.30	35.89	4.41	0.58	0.38	0.26	0.36	0.09	1.69	3.35
	Ripe bananas.....	6	4.46	35.12	30.78	4.34	.60	.39	.24	1.15	.02	.70	2.41
1065	Full ripe bananas.	13	10.04	28.02	23.63	4.40	.59	.39	.24	1.07	.10	.56	2.33
	Pulp:												
	Green bananas....	0	0.00	59.70	43.54	16.15	.54	.32	.62	.46	.22	12.86	14.85
	Ripe bananas.....	6	4.46	60.42	44.07	16.36	.57	.31	.59	9.92	.62	3.27	4.70
	Full ripe bananas.	13	10.04	61.94	47.06	14.88	.53	.30	.58	8.65	3.31	.47	1.70
	Peel:												
	Green bananas....	0	0.00	40.15	35.23	4.92	.55	.35	.37	.28	.08	1.98	3.81
	Ripe bananas.....	5	3.33	36.35	31.66	4.69	.56	.35	.32	1.14	.18	.92	2.73
1066	Full ripe bananas.	13	9.43	28.28	23.64	4.64	.54	.34	.30	1.35	.07	.55	2.29
	Pulp:												
	Green bananas....	0	0.00	59.85	43.22	16.63	.53	.31	.58	.38	.10	13.50	15.51
	Ripe bananas.....	5	3.33	60.32	43.35	17.05	.54	.30	.70	4.64	5.66	3.92	5.43
	Full ripe bananas.	13	9.43	62.29	47.16	15.14	.51	.29	.72	9.06	3.16	.47	1.72
	Peel:												
	Green bananas....	0	0.00	40.35	35.56	4.79	.55	.35	.36	.39	.05	1.78	3.53
	Ripe bananas.....	5	3.17	36.26	31.66	4.60	.55	.35	.29	1.02	.24	.85	2.57
1067	Full ripe bananas.	13	8.90	28.06	23.44	4.62	.55	.35	.34	1.26	.05	.50	2.34
	Pulp:												
	Green bananas....	0	0.00	59.65	43.02	16.63	.55	.32	.82	.25	.30	13.38	15.54
	Ripe bananas.....	5	3.17	60.57	43.53	17.04	.58	.30	.80	4.46	5.55	3.97	5.42
	Full ripe bananas.	13	8.90	63.04	47.53	15.51	.54	.30	.78	8.27	4.22	.49	1.75
	Peel:												
	Green bananas....	0	0.00	43.44	38.55	4.89	.57	.37	.32	.42	.07	1.58	3.62
	Ripe bananas.....	7	3.98	38.12	33.41	4.71	.58	.37	.28	1.13	.04	.79	2.52
1068	Full ripe bananas.	13	8.02	31.52	26.91	4.61	.58	.38	.29	1.13	.05	.56	2.36
	Pulp:												
	Green bananas....	0	0.00	56.56	40.67	15.89	.48	.28	.68	.16	.24	12.73	14.83
	Ripe bananas.....	7	3.98	57.90	41.73	16.17	.52	.28	.66	8.30	1.15	3.98	5.59
	Full ripe bananas.	13	8.02	60.46	45.30	15.16	.48	.27	.64	5.99	4.12	.62	1.92

A statement of the average composition of the pulps of the four bunches of bananas as analyzed, summarized from Table III, is given in Table IV.

TABLE IV.—*Average composition in terms of percentage of pulp of bananas at different stages*

Stage.	Percentage of whole fruit.	Water.	Solids.	Ash.	Alkalinity of ash.	Protein, N×6.25.	Reducing sugars as invert.	Sucrose.	Starch.
Green pulp	58.94	72.29	27.71	0.89	0.52	1.23	0.53	0.41	22.26
Ripe pulp	62.12	72.76	27.84	.92	.50	1.17	11.54	5.39	6.34
Very ripe pulp	68.14	75.50	24.50	.84	.47	1.10	12.89	5.98	.82

FOURTH EXPERIMENT

In the fourth experiment lots of bananas of the same initial ripeness and taken from the same stem were analyzed successively during ripening. The rate of respiration of each lot was determined immediately before its analysis. It was thus possible to correlate the chemical

changes with the changes in rate of respiration. The results show that the most rapid starch hydrolysis occurs at the time when the respiration rate is greatest. The following is a detailed account of the work.

The fruits from a large bunch of green bananas were cut from the stem by first cutting off the "hands" and then separating the individual fruits, finally removing from each fruit the small piece of adhering stem, cutting where the stem was most constricted, as in sampling bananas in previous studies. A few drops of sticky, turbid juice oozed from each banana, and care was taken not to allow the fruit surfaces to become wet with it. Fruits from the inside and the outside of each "hand" were divided as evenly as practicable into nine different lots, each lot receiving fruits from all parts of the bunch, and consisting of from 13 to 17 fruits. The lots were weighed and placed in the humidity chamber on the afternoon of May 1. The analysis of the first lot was made upon the afternoon of the following day, immediately after the determination of its respiratory activity. The remaining lots were successively analyzed at intervals of from one to three days, except the ninth lot, which was kept longest, and spoiled. See Table V.

TABLE V.—*Composition of detached bananas during ripening in the humidity chamber*

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGES OF PEEL AND PULP OF THE BANANAS BEFORE AND AFTER RIPENING

Date.	Part of fruit.	Interval in humidity chamber.	Loss in weight in humidity chamber.	Percentage of whole fruit.	Composition of peel or pulp.				
					Water.	Solids.	Alcohol-insoluble solids.	Total sugar as invert.	Starch.
	PEEL.								
1912.		*Days.*							
May 2	Green bananas	1	1.66	38.22	89.46	10.54	6.84	1.39	3.28
3	Slightly yellowing bananas	2	2.47	37.54	88.61	11.39	8.52	2.16	2.93
4	....do	3	3.07	35.41	88.04	11.30	6.18	2.91	2.11
6	Yellow bananas	5	6.48	32.57	86.84	13.16	6.09	3.64	1.63
8	.....do	7	7.83	30.88	85.90	14.10	6.67	3.89	1.69
10	.....do	9	8.39	29.36	85.08	14.92	7.08	3.98	1.82
13	.....do	12	10.56	27.95	84.25	15.75	7.57	4.07	1.88
16	Brown bananas	15	11.32	26.26	83.32	16.68	8.12	4.38	1.84
	PULP.								
2	Green bananas	1	1.66	61.77	72.47	27.53	21.95	4.35	19.01
3	Slightly yellowing bananas	2	2.47	62.45	72.37	27.63	16.46	9.65	13.96
4	.... do	3	3.07	64.58	72.44	27.56	11.04	15.00	8.02
6	Yellow bananas	5	6.48	67.42	73.26	26.74	5.82	19.65	3.66
8	....do	7	7.83	69.12	73.45	26.55	5.89	20.68	1.73
10	.....do	9	8.39	70.65	74.63	25.37	3.33	20.61	1.15
13	.....do	12	10.56	72.05	75.72	24.28	2.88	20.05	.87
16	Brown bananas	15	11.32	73.74	77.02	22.98	3.17	18.21	1.01

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS

	PEEL.								
May 2	Green bananas	1	1.66	37.59	33.63	3.96	2.57	0.53	1.23
3	Slightly yellowing bananas	2	2.47	36.01	32.44	4.17	3.12	.80	1.07
4	.... do	3	3.07	34.11	30.23	3.88	2.11	.99	.72
6	Yellow bananas	5	6.48	30.36	26.45	4.01	1.86	1.11	.50
8	.....do	7	7.83	28.46	24.45	4.01	1.90	1.11	.48
10	.....do	9	8.39	26.90	22.89	4.01	1.90	1.07	.49
13	.....do	12	10.56	25.00	21.06	3.94	1.89	1.02	.47
16	Brown bananas	15	11.32	23.29	19.40	3.89	1.89	1.02	.43

TABLE V.—*Composition of detached bananas during ripening in the humidity chamber*—Continued

COMPOSITION EXPRESSED IN TERMS OF PERCENTAGE OF THE WHOLE GREEN BANANAS—continued

Date.	Part of fruit.	Interval in humidity chamber.	Loss in weight in humidity chamber.	Percentage of whole fruit.	Composition of peel or pulp.				
					Water.	Solids.	Alcohol-insoluble solids.	Total sugar as invert.	Starch.
	PULP.								
1912.		*Days.*							
May 2	Green bananas	1	1.66	60.74	44.02	16.72	13.33	2.65	11.55
3	Slightly yellowing bananas	2	2.47	60.91	44.08	16.83	10.03	5.88	8.50
4	.....do	3	3.67	62.21	45.06	17.15	6.87	9.33	5.36
6	Yellow bananas	5	6.48	63.05	46.19	16.86	3.67	12.39	2.32
8	.....do	7	7.83	63.70	46.79	16.91	2.48	12.79	1.10
10	.....do	9	8.39	64.72	48.30	16.42	2.03	13.34	.74
13	.....do	12	10.56	64.44	48.79	15.65	1.86	12.91	.56
16	Brown bananas	15	11.32	65.39	50.36	15.03	2.07	11.91	.65

In order to determine correctly the respiration rate at intervals on ripening, it was necessary to collect the carbon dioxid evolved during relatively short periods.

The bananas were placed in a tubulated desiccator, kept in the dark at 20° C., and a rapid current of air passed through. The air was first freed from carbon dioxid by passing it through a long wide glass tube filled with soda lime. The carbon dioxid evolved by the bananas was collected by drawing the air from the desiccator through a Reiset scrubbing tube containing soda solution. The Winkler method of titration of the absorbed carbon dioxid was employed.

In operating the flask of the Reiset apparatus was charged with a mixture of 500 c. c. of distilled water and 100 c. c. of an approximately normal solution of sodium hydroxid. The distilled water had previously been well aerated to remove carbon dioxid, and the titer of the soda solution (after addition of barium chlorid to precipitate carbonates) was known. After mixing, the Reiset tube was inserted, connection made with the desiccator containing the bananas, and suction applied. After absorption, the contents of the Reiset apparatus were washed into a large precipitating jar with aerated distilled water, excess of barium chlorid added, and the solution titrated with normal hydrochloric acid, using phenolphthalein as indicator. Each cubic centimeter of normal alkali consumed equals 0.022 gm. of carbon dioxid. In titrating it was necessary to admit the acid under the surface of the solution and stir well to avoid escape of carbon dioxid freed by local momentary excess of acid. As the amounts of carbon dioxid expected were approximately known it was found convenient to use a slight excess of solution of barium chlorid of such strength that each cubic centimeter decomposes 1 c. c. of normal sodium carbonate.[1]

Lead tubes were used in leading the air to and from the desiccator. The air entered it near the top and was withdrawn from near the bottom.

[1] For a criticism of volumetric methods of estimation of carbon dioxid, see Küster (15).

At the start of each experiment a current of carbon-dioxid-free air was led through the desiccator for about half an hour at the rate of from 1 to 2 liters per minute, this interval constituting a fore period during which the carbon dioxid was not collected. The absorber was then inserted in the train and the air passed through at the same rate as during the fore period. A water gauge inserted between the desiccator and the Reiset tube served to indicate the relative rate of flow. After an interval of from one to two hours the absorption apparatus was replaced by a freshly filled Reiset tube and the carbon dioxid collected for a second interval. The rate of respiration was thus determined during two successive intervals. In general, well-agreeing duplicates were obtained.

The figures showing the results of the study of the rate of respiration are given in Table VI. At the beginning the bananas were just on the point of turning yellow and were much more active (106 mg. of carbon dioxid per kg. hour) than the green fruit used in the calorimeter studies (30 mg. and 30 mg., respectively). The respiration was most intense (146 mg. per kg. hour) when the rate of transformation of starch was greatest. It then slowly slackened, reaching at the end 91 mg. per kg. hour.[a] As the rate of respiration of each sample analyzed was determined immediately before its analysis, the total amount of carbon dioxid evolved during the ripening period, 3.776 per cent of the original bananas, was easily estimated by a summation process from the data given in Table VI.

TABLE VI.—*Rate of respiration of detached bananas ripening in the humidity chamber*

Date.	Description.	Number of fruits.	Original weight.	Carbon dioxid collected	Intervals for which collected.	Respiration rate.[b]	Average respiration rate.[b]	Interval from middle of one collection period to middle of next	Average rate of respiration from middle of one period to middle of next.[b]
			Gm.	*Gm.*	*Hours.*	*Gm*	*Gm.*	*Hours.*	*Gm.*
May 2	Wholly green, but on the point of turning yellow.	14	2.410	0.3344	1.33	0.104	0.106	24	0.126
				.4570	1.75	.108			
3	Bananas just beginning to turn yellow, sample ripening uniformly	25	2.552	.464	1.25	.145	.146	24	.142
				.656	1.75	.147			
4	Fruit yellower than on day previous, much green still present	14	2.506	.857	2.50	.136	.138	48	.128
				.669	1.92	.139			
6	Fully yellowed, but many specimens are green at the tips	13	2.300	.337	1.25	.117	.117	50	.113
				.075	2.53	.110			
8	Fully yellowed	16	2.835	.526	1.70	.109	.110	48	.108
10	Fruits beginning to brown slightly at the surfaces	17	2.919	.352	1.12	.111	.106		
				.453	1.47	.106			
11	Skins brown, much yellow still present	14	[c] 2.520	.383	1.25	.105	.101	70	.104
16	Skins almost entirely brown, a little yellow present at ribs	14	[d] 2.535	.403	2.00	.101	.101	74	.096
				.374	1.78	.092			
				.376	1.78	.091			

[a] In the two calorimeter studies the respiration rate of the bananas reached maxima of 150 and 200 mg. per kg. hour, respectively, and the respiratory activities at the end were 110 and 100 mg. per kg. hour, respectively.

[b] Grams carbon dioxid per kg. per hour.

[c] Weight used in calculating was 1,990 gm., three fruits having been rejected whose weight calculated to the original weight was subtracted from the original weight of the sample.

[d] Weight used in calculating was 2,310 gm., one fruit having been rejected, its weight calculated to original and subtracted.

The analytical data are given in Table V. The study of the detached fruit permitted a longer period of observation than when bananas attached to the stem were used. The rate of transformation of starch into soluble carbohydrates was very rapid at first—32.11 gm. per kg. per day. It then increased to 34.9 gm. per kg. per day. Starch hydrolysis, so far as revealed by analysis, nearly ceased six days before the end of the life history of the bananas. The analytical data confirm the facts developed in the earlier experiments.

DISCUSSION OF RESULTS

As the result of the foregoing studies, the author is in a position to state more exactly than has heretofore been possible the nature and extent of the changes in the composition of bananas during ripening. The most conspicuous change is the long-recognized conversion of starch into sugars. It is most rapid while the fruits are turning from green to yellow. During this period the respiration rate increases manyfold, becoming greatest at the time when the rate of starch hydrolysis is most rapid. Starch hydrolysis then gradually slackens, later ceasing altogether. The respiration rate, too, becomes slower, but still remains far more active than in the green fruit. Next to the starch and respiration changes, most conspicuous are those of water. The peel loses, while the pulp gains water steadily. The respective losses and gains in water of the peel and the pulp on ripening, expressed in terms of the original green bananas, are summarized in Table VII.

TABLE VII.—*Percentage of losses and gains in water of peel and pulp of bananas on ripening*

Experiment No.	Place of ripening.	Loss of water in peel.	Actual gain of water in pulp.	Gain of water in pulp corrected for water formed and absorbed in physiological processes.
1	Calorimeter	5.01	1.64	2.4
2	do	10.45	3.02	3.5
3	Humidity chamber	12.26	3.52	
4	do	11.59	3.94	
		12.12	4.51	
		11.64	4.63	
		14.23	6.34	6.135

In the first, second, and fourth experiments it is possible to show how much water is formed or absorbed by the pulp in physiological processes. The water formed in respiration can easily be calculated if formed in consequence of the complete combustion of carbohydrates and if the amount of carbon dioxid evolved on ripening in consequence of this combustion is known. The respiratory quotient and the thermal quotient determined by the Office of Nutrition Investigations for ripening bananas (16) agree in showing that the carbon dioxid evolved on normal ripening is due solely to the complete combustion of carbohydrates.

We are therefore justified in calculating the water formed by the equation $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. From the water so formed is subtracted the water absorbed in the saccharification of starch. See Table VIII.

TABLE VIII.—*Percentage of water formed by the pulp of bananas in physiological processes*

Experiment No.	Carbon dioxid found.	Calculated water formed from equation I.	Loss of carbohydrate found on ripening in —a		Calculated water proportioned according to carbohydrate loss in—		Calculated water absorbed in pulp in starch hydrolysis.
			Peel.	Pulp.	Peel.	Pulp.	
1	1. 334	0. 546	0. 46	0. 88	0. 188	0. 358	1. 14
2	2. 256	. 923	. 41	1. 43	. 205	. 718	1. 23
4	3. 776	1. 545	. 40	2. 84	. 190	1. 355	1. 16

a Probably slightly larger than actual on account of failure to completely estimate maltose. See p. 189.

In the first two experiments absorption of water amounting to 0.782 and 0.512 per cent occurred as a net result of respiration and starch hydrolysis. In the fourth experiment, where the bananas became overripe, the water formed in respiration was greater by 0.195 per cent than that absorbed in starch hydrolysis.

The increases of water in the pulp during ripening are all derived from the peel, except when bananas become overripe, when the water formed in respiration may more than balance the water absorbed in starch hydrolysis. From the quantity of sugar formed in the pulp it is evident that the osmotic pressure of the pulp must undergo a marked increase, with a corresponding decrease of vapor pressure, during the ripening of the fruit. A possible operating cause of the water transfer from peel to pulp is obvious.

From a knowledge of the carbon dioxid formed in respiration and knowing from the calorimeter data that carbon dioxid results from the complete combustion of carbohydrates, it can be determined whether or not the carbohydrates consumed in respiration were accurately made known from the analyses. Carbohydrate losses found by analysis contrasted with the expected losses from the calorimeter data are shown in Table IX.

TABLE IX.—*Comparison of carbohydrate losses with the expected losses from carbon dioxid in pulp of ripening bananas*

Carbohydrate losses found (expressed as hexose).	Expected losses from carbon dioxid formed in respiration.
Per cent.	*Per cent.*
1. 34	0. 91
1. 84	1. 54
3. 24	2. 58

By analysis somewhat greater losses appear than indicated from the calorimeter data. It is not improbable that the small differences are due to analytical error.

SUMMARY

(1) The usual carbohydrate changes—saccharification of starch, with formation of sucrose and invert sugar, and consumption of sugars in respiration—proceeded with uniformity in bananas of different bunches.

(2) The period of most rapid respiration corresponded closely with that of most rapid starch hydrolysis.

(3) The quantities of ash, protein, and ether extract underwent but slight changes during the ripening of the bananas. Pentosans decreased markedly in the pulp, but remained little changed in the peel.

(4) Analyses of the peel and pulp of ripening bananas showed a steady transfer of water from peel to pulp during ripening.

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ASSIMILATION OF COLLOIDAL IRON BY RICE

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INTRODUCTION

Previous work [1] at the Porto Rico Agricultural Experiment Station has shown that pineapples and upland rice grown on moderately or strongly calcareous soils are affected with chlorosis and that the failure of these plants to make a successful growth on such soils seems to be due wholly or in part to a diminished assimilation of iron. A determination of the forms of iron available to rice is therefore important. An experiment on the assimilation of colloidal iron is reported here, as it bears on this problem as well as on the properties of plant roots.

MATERIALS AND METHODS OF THE EXPERIMENT

The availability of colloidal iron was compared with that of ferric chlorid by growing upland rice in a nutrient solution. Water cultures were used in this work, as it is impossible to tell in what form the iron may be present in soil cultures. To prevent precipitation of the colloidal iron by other salts of the nutrient solution the iron was put in one flask (flask B) and the other salts in a second flask (flask A). The plants were grown with part of their roots in each flask.

The seeds were germinated over distilled water until they had developed two or more roots. Two plants were grown in each pair of flasks. Two hundred c. c. Erlenmeyer flasks of Jena glass with their necks joined together by surgeon's tape were used. The formula for the nutrient solution used in flasks A, which gave excellent results with rice in previous work, was as follows:

KNO_3	0.1017 gm.	$CaCl_2$	0.05 gm.
KH_2PO_4	.0714 gm.	$MgCl_2$	.05 gm.
$NaNO_3$	.2143 gm.	H_2SO_4	.5 c. c. N/10.
Na_2SO_4	.0315 gm.	Distilled water	1,000 c. c.

The plants were changed to fresh solutions every few days.

The colloidal iron used was the ordinary dialyzed iron. It contained 0.0383 gm. of Fe and 0.0058 gm. of Cl_2 per c. c. After salting out the colloidal iron with potassium sulphate, the filtrate was examined and found to contain the following, calculated as grams per c. c. of the original dialyzed iron: Cl_2, 0.00436 gm.; Fe, hardly a reaction with potassium

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sulphocyanate; acidity, equivalent to 0.002 gm. of Cl_2 from hydrochloric acid; ammonia, none with Nessler's reagent. The dialyzed-iron preparation after dilution was dialyzed for 24 hours with a parchment membrane without any iron appearing in the dialyzate.

While no soluble or ionized iron appeared in these tests we must assume that some existed in the dialyzed-iron preparation because of the high chlorin content. The nonappearance of iron in the dialyzate after dialysis and in the filtrate after salting out the iron was probably due to adsorption by the colloid or precipitate and to the strong hydrolysis that dilute ferric chlorid undergoes.[1] From the chlorin content it appears that about one-twelfth of the iron could have been present as ferric chlorid, but we can hardly assume that it was there as such or that a certain quantity of ferric chlorid in a colloidal-iron solution would act the same as in a simple aqueous solution. We can simply assume that the soluble iron bore some proportion to the quantity of chlorin. In the following tests, then, a low availability of the dialyzed-iron preparation is not proof of the assimilation of colloidal iron.

RESULTS OF EXPERIMENTS

EXPERIMENT I.—In a preliminary test the plants were grown for 42 days. The dialyzed iron was used at the rate of 10.5 parts of Fe per 100,000 parts of water for the first 10 days and at the rate of 1.05 parts of Fe per 100,000 for the remaining 32 days. The ferric chlorid was used at the rate of 0.41 part of Fe per 100,000. The results are given in Table I.

TABLE I.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment I*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops.	Gain over no-iron plants
			Gm.	*Gm*	*Gm*
5–8	Nutrient solution without Fe.....	Distilled water.....	1.03	0.17	.....
9–12	.....do	Dialyzed iron.....	3.25	.54	0.37
13–16	.....do.....	Ferric chlorid.....	4.02	.67	.50
1–4	Nutrient solution + $FeCl_3$.....	Distilled water.....	5.82	.95	.78

The preparation of dialyzed iron had a certain availability, but relatively large amounts were less effective than the smaller quantity of ferric chlorid.

EXPERIMENT II.—In a second experiment the dialyzed iron and ferric chlorid were both used to furnish 0.4 gm. of Fe per 100,000 c. c. of water. The plants were grown for 59 days. The results are given in Table II.

[1] This was borne out by the following test: To 1 c. c. of dialyzed iron 0.00275 gm. of Fe from $FeCl_3$ was added; the solution was made to 100 c. c. and the colloidal iron salted out by K_2SO_4. The filtrate, tested for soluble iron (colorimetric method with KSCN), showed 0.00245 gm. of Fe had been lost. Of the 0.00245 gm. of Fe lost 0.00056 gm. was lost by adsorption by the precipitate. Precipitation by hydrolysis by water alone caused a loss of 0.00025 gm. of Fe, and hydrolysis in the presence of K_2SO_4 caused a loss of 0.00189 gm. of Fe.

TABLE II.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment II*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops.	Average oven-dry weight of tops.	Gain over no-iron plants.
			Gm.	*Gm.*	*Gm.*	*Gm.*
1–4	Nutrient solution without Fe.	Distilled water	3.38	0.60	0.60	.....
5–8	do	0.4 gm. of Fe per 100,000 c. c. from dialyzed iron.	4.41	.76	.....	.....
9–12	do	do	4.83	.79	.....	.....
13–16	do	do	4.71	.78	.78	0.18
17–20	do	0.4 gm. of Fe per 100,000 c. c. from FeCl3.	6.40	1.07	.....	.....
21–24	do	do	5.61	1.05	.....	.....
25–28	do	do	7.82	1.31	1.14	.54

In this experiment, where equivalent and small quantities of iron were used, the dialyzed-iron preparation appeared to have an availability of about three-tenths that of the ferric chlorid.

EXPERIMENT III.—A third series was conducted, using equivalent small quantities of iron, ten times this amount of iron from dialyzed iron, and twice this quantity of iron from ferric chlorid. The results are given in Table III.

TABLE III.—*Growth of rice with dialyzed iron and ferric chlorid—Experiment III*

Nos. of flasks.	Solution in flasks A.	Solution in flasks B.	Green weight of tops.	Oven-dry weight of tops	Average oven-dry weight of tops	Gain over no-iron plants.	Average oven-dry weight of roots in flasks A.	Average oven-dry weight of roots in flasks B
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1–5	Nutrient solution without Fe	Distilled water	3.93	0.73	.....	.....	.....	.....
6–10	do	do	3.47	.69	0.71	.....	0.144	0.055
11–15	do	Fe from dialyzed iron, 0.4 gm. per 100,000 c.c.	4.35	1.06	.....	.....	.....	.....
16–20	do	do	3.75	.93	1.00	0.29	.201	.215
21–25	do	do	8.78	1.55	.....	.....	.....	.....
26–30	do	do	5.77	1.05	1.30	.59	.212	.194
31–35	do	Fe from FeCl3, 0.4 gm per 100,000 c. c.	5.51	1.00	.....	.....	.....	.....
36–40	do	do	6.69	1.24	1.12	.41	.217	.077
41–45	do	Fe from FeCl3, 0.8 gm. per 100,000 c. c.	8.90	1.57	.....	.....	.....	.....
46–50	do	do	9.62	1.55	1.50	.85	.270	.174
51–55	Nutrient solution + FeCl3.	Distilled water	17.92	2.81	.....	.....	.....	.....
56–60	do	do	20.63	3.73	2.97	2.26	.511	.290

Flasks Nos. 26 to 30 did not agree well with Nos. 21 to 25, so it is possible that the average of 1.30 gm. is too low. It is apparent from the preceding tests that the dialyzed iron was much less available than the ferric chlorid. On the basis of the Fe content, the dialyzed iron had to be present in at least five times the amount of ferric chlorid to produce the same yield.

The percentages of iron in the dry substance of the tops was determined in the plants from Experiment III. The percentages varied from

0.035 to 0.037 per cent of Fe_2O_3. Since the samples were small, it is felt that the figures merely show that practically the same percentages of iron were present in all the plants. Thus, the quantities of iron in the different lots of plants would vary as their dry weights.

In all the experiments the plants which received no iron in either flasks A or flasks B were strongly chlorotic, the chlorosis commencing about 6 to 10 days after the plants were put in the solutions. The plants receiving dialyzed iron or ferric chlorid in flasks B were also strongly chlorotic, although they were somewhat greener than the check plants without any iron, and the chlorosis was later in appearing. The plants which had the ferric chlorid added to the other nutrient salts in flasks A were of a normal green color.

The root development varied greatly in the different flasks. In all the flasks A which contained the complete nutrient solution without iron the root development was good, the main roots being long, with numerous long laterals. The roots in flasks B which contained only distilled water made very little growth and had few laterals. The roots in flasks B which contained only ferric chlorid made even less growth than those in distilled water. As soon as the roots penetrated the ferric-chlorid solution, the root tip appeared killed and no roots penetrated the solution for any distance. The roots in flasks B which contained dialyzed iron developed much better than in distilled water. During the latter stages of growth particularly a few heavy roots developed in the dialyzed iron, but these roots carried very few laterals. The oven-dry weights of the roots in flasks B given in Table III do not really give a comparison of the root developments in the solutions, for the reason that the weights of the roots in flasks B, Nos. 31–60, were made up chiefly of root "stubs"—heavy roots which started out from the plants but did not develop in the distilled water or ferric-chlorid solution. The larger the top growth, dependent on the amount of iron obtained, the more root "stubs" developed. For instance, flasks B, Nos. 41–46 and Nos. 36–50, had 0.174 gm. of roots, while Nos. 31–35 and Nos. 36–40 had but 0.077 gm. of roots. As a matter of fact, the root development in the solution was less in the first case than in the second.

DISCUSSION OF RESULTS

The poor development of the roots in the flasks B was, of course, due to the well-established toxicity of unbalanced solutions—in this case of single salts or distilled water.[1] Because of the injury resulting from the iron solutions it is impossible to draw a very sharp conclusion concerning the assimilation of colloidal iron. Although the roots developed better in

[1] True holds the injury from ordinary distilled water "but a special case of the general type of injury wrought on cells by unbalanced solutions."—True, R. H. The harmful action of distilled water. In Science, n. s., v. 39, no. 999, p. 395. 1914.

the dialyzed iron than in the ferric chlorid,[1] they assimilated less iron, even in solutions containing three to five times more dialyzed iron than ferric chlorid. It seems probable that the small amount of iron obtained from the dialyzed iron was not colloidal iron but soluble iron. It is true that the tests made of the dialyzed-iron preparation revealed little or no ionized iron, but we must assume the presence of some soluble iron from the chlorin content of the preparation.

In view of the low availability of the dialyzed-iron solution it would seem that the often-mentioned but questionable acid excretion of roots was not operative, at least not in this unbalanced solution. With respect to fineness of division and contact with the roots the colloidal iron was especially favorable for assimilation by an acid if the roots had excreted such. An apparent objection to the conclusion that colloidal iron is not assimilable is the fact that dialyzed iron is sometimes used in nutrient solutions and that in Von Crone's nutrient solution ferric phosphate is employed. In such solutions, however, it is not at all certain that the plants utilize insoluble iron compounds. In fact, from further work in progress it appears that rice at least is capable of assimilating only soluble iron in nutrient solutions.

Aside from the question of the assimilation of colloidal iron, the preceding test is of interest in connection with the study of unbalanced solutions. The fact that while some roots of the plant were developing well in the balanced solution of the flasks A other roots of the same plant were injured by the distilled water or ferric chlorid of the flasks B shows that in case the toxicity of certain salt solutions or ordinary distilled water is due to injury in the root cells by extraction of other electrolytes,[2] this extraction takes place faster than the electrolytes can be supplied from other parts of the plant. For in this case the roots in flasks A had an abundance of the electrolytes to draw on.

The idea that the toxicity of single-salt solutions is due to or accompanied by the penetration of the salts finds confirmation in the preceding experiments. It is evident from Experiment III that the stronger the ferric chlorid solution the less the root development in the solution, but the greater the amount of iron absorbed (as shown by the top growth which was dependent on iron absorbed). Osterhout[3] has shown by electrical conductivity measurement that single-salt solutions penetrate cells of the kelp, Laminaria.

[1] The reduction of the toxicity of distilled water by colloids and finely divided solids has been frequently noted.

[2] True, R. H. Loc. cit.

[3] Osterhout, W. J. V. The permeability of protoplasm to ions and the theory of antagonism. *In* Science, n. s., v. 35, no. 890, p. 112-115, 1912.

SUMMARY

The work reported would seem to show that rice can not assimilate colloidal iron. It is believed that the iron obtained from the dialyzed-iron preparation was soluble iron.

It is apparent that the toxicity of ordinary distilled water or ferric-chlorid solutions for plant roots can not be overcome by supplying other roots of the same plant with a balanced solution.

The toxicity of the ferric-chlorid solution was accompanied by the penetration of iron into the root and transportation to the leaves.

COLORING MATTER OF RAW AND COOKED SALTED MEATS

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INTRODUCTION

The red color of fresh lean meat, such as beef, pork, and mutton, is due to the presence of oxyhemoglobin, a part of which is one of the constituents of the blood remaining in the tissues, while the remainder is a normal constituent of the muscles. When fresh meat is cooked or is cured by sodium chlorid, the red color changes to brown, owing to the breaking down of the oxyhemoglobin into the two constituents, hematin, the coloring group, and the proteid, globin.

On the other hand, when fresh meat is cured by means of a mixture of sodium chlorid and a small proportion of potassium nitrate, or saltpeter, either as a dry mixture or in the form of a pickle, the red color of the fresh meat is not destroyed during the curing process, the finished product having practically the same color as the fresh meat. Neither is the red color destroyed on cooking, but rather is intensified.

The practical value of saltpeter in the curing of meats is so well known that its use for this purpose may be said to have become practically universal; such use is not confined to the commercial meat-packing industry, but it is used in the home curing of meats as well.

It is only within comparatively recent years, however, that anything very definite has been known concerning the nature of the color of salted meats or the process of the color formation. The work which is reported in this paper was undertaken for the purpose of obtaining more complete information concerning the color of raw and cooked salted meats.

HISTORICAL SUMMARY

Weller and Riegel (1897),[1] in the examination of a number of samples of American sausages, obtained a red coloring matter on extracting the samples with alcohol and other solvents, which color they concluded to be in some manner due to the action of the salts used in curing upon the natural color of the meat. On account of similarity of spectra, this color was considered to be methemoglobin.

Lehmann (1899) observed that when fresh meat was boiled in water containing nitrites and free acid or in old meat broth the surface of the meat turned bright red in color, in contrast to the brown color which fresh meat takes on when boiled in water free from nitrites. The addi-

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 225.

Journal of Agricultural Research,
Dept of Agriculture, Washington, D. C.

Vol. III, No. 3
Dec. 15, 1914
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tion of nitrates to the water did not cause the production of the red color on the surface of the meat on boiling. This color was found to be soluble in alcohol and ether and to give a spectrum showing an absorption band just at the right of the D line, and a second band, often poorly defined, at the left of the E line. On standing, the color of the solution changed to brown and gave the spectrum of alkaline hematin.

A color with similar properties was obtained on extracting hams and various kinds of sausages with alcohol and other solvents. The author named this coloring matter "hæmorrhodin."

Kisskalt (1899) studied the production of red color in fresh meats on cooking and found that this color appeared when meat was cooked in bouillon which was several days old, or in water containing nitrites and free acid. Meat boiled in water to which saltpeter had been added did not take on the red color; but, on the other hand, if the meat was first allowed to stand several days in contact with saltpeter and then boiled, the red color appeared.

Haldane (1901) made an extensive study of the color of cooked salted meat, which color he concluded to be due to the presence of the nitric-oxid hemochromogen resulting from the reduction of the coloring matter of the uncooked meat, or nitric-oxid hemoglobin (NO-hemoglobin). This color was found to be the same as that resulting from the boiling of fresh meat in water containing nitrites and free acid. It exhibited a spectrum showing a distinct band just at the right of the D line and a faint band a trifle to the left of the E line. The color was found to be soluble in alcohol and in ether and to be quite resistant to the action of reducing agents.

The color of uncooked salted meats was found to be soluble in water and gave a spectrum characteristic of NO-hemoglobin. The formation of the red color in uncooked salted meats is explained by the action of nitrites in the presence of a reducing agent and in the absence of oxygen upon hemoglobin, the normal coloring matter of fresh meats.

Orlow (1903) states that the red color of sausages is due to the action upon the color of the fresh meat of the nitrites resulting from the reduction of the saltpeter used in the process of manufacture.

The present author (1908) studied the action of saltpeter upon the color of meat and found that the value of this agent in the curing of meats depends upon its reduction to nitrites and nitric oxid, with the consequent production of NO-hemoglobin, to which compound the red color of salted meats is due. Saltpeter, as such, was found to have no value as a flesh-color preservative.

Glage (1909) is the author of a pamphlet concerning practical methods for obtaining the best results from the use of saltpeter in the curing of meats and in the manufacture of sausages. The fact that the value of saltpeter as a flesh-color preservative is dependent upon the reduction of the nitrate to nitrite is recognized, and directions are given for the

partial reduction of the saltpeter to nitrites by heating the dry salt in a kettle before it is to be used. It is stated that this partially reduced saltpeter is much more efficient in the production of color in the manufacture of sausage than is the untreated saltpeter.

Humphrey Davy in 1812 (cited by Hermann, 1865) and Hoppe-Seyler (1864) noted the action of nitric oxid upon hemoglobin, but it appears that Hermann (1865) was the first to furnish us with much information as to the properties of this derivative of hemoglobin. He prepared NO-hemoglobin by first passing hydrogen through dog's blood until spectroscopic examination showed that all of the oxyhemoglobin had been reduced to hemoglobin, then saturating the blood with pure nitric oxid prepared from copper and nitric acid, and finally again passing hydrogen through the blood to remove all traces of free nitric oxid. It was observed that the spectrum of hemoglobin had changed to one showing two bands in practically the same position as those of oxyhemoglobin. The blood saturated with nitric oxid was found to be darker in color than either arterial blood or that saturated with carbon monoxid, and on exposure to air or on treatment with ammonium sulphid it proved to be as stable as carbon-monoxid hemoglobin.

NO-hemoglobin is mentioned but briefly in most of the recent texts on physiological or organic chemistry as being a hemoglobin derivative of but little practical importance. Abderhalden (1911) and Cohnheim (1911), however, describe this compound quite fully.

EXPERIMENTS WITH NO-HEMOGLOBIN

The following studies on NO-hemoglobin were conducted by the writer.

Formation.—Nitric oxid was prepared by treating copper foil with concentrated nitric acid, and the production of the gas was carried on long enough to free the apparatus from higher oxids of nitrogen before conducting gas into the solution of hemoglobin. A simple apparatus was arranged so that the sample of blood or of hemoglobin to be treated was first saturated with pure hydrogen, then with nitric oxid, and finally again with hydrogen to remove all free nitric oxid. Great care was exercised to exclude air from the apparatus, since the presence of even a small amount of free oxygen results in the oxidation of nitric oxid to nitrogen peroxid, with the consequent production of nitrous and nitric acids, which act upon hemoglobin to form methemoglobin.

NO-hemoglobin was prepared, in the manner described above, both from defibrinated blood and from a solution of oxyhemoglobin prepared by Hoppe-Seyler's method. It was found, as a rule, that if a solution of oxyhemoglobin or of defibrinated blood was treated with nitric oxid, with all precautions to exclude free oxygen, the NO-hemoglobin usually contained a small amount of methemoglobin. This fact has been noticed by other investigators and is due to the union of the loosely

bound oxygen of the oxyhemoglobin with the nitric oxid to form nitrogen peroxid, which, as has just been noted, acts upon the hemoglobin to form methemoglobin.

Some of the earlier investigators have found that small quantities of oxyhemoglobin could be reduced to hemoglobin by means of hydrogen; but it has been the experience of the writer that, when working with any considerable quantity of oxyhemoglobin, practically no reduction took place even after passing a current of hydrogen through an oxyhemoglobin solution for several hours. In practice it was found best to reduce oxyhemoglobin to hemoglobin by means of hydrazin hydrate before saturation with nitric oxid.

PROPERTIES.—NO-hemoglobin in concentrated solution has a dark cherry-red color; in dilute solutions it has a light cherry-red color, in contrast to the bright-red color of oxyhemoglobin or to the purple-red color of hemoglobin in solutions of the same concentration. In solutions free from methemoglobin NO-hemoglobin is quite stable, and solutions of the compound have been kept in a refrigerated room at 32° to 35° F. for several weeks without apparent change. On boiling a solution of NO-hemoglobin a brick-red precipitate is formed, in contrast to the dark-brown precipitate formed on boiling a solution of oxyhemoglobin or hemoglobin.

NO-hemoglobin shows a characteristic spectrum consisting of a heavy band just at the right of the D line and a somewhat lighter and wider band a trifle to the left of the E line (fig. 1). These absorption bands occupy practically the same positions as those of oxyhemoglobin, but are distinguishable from the latter in solutions of the same concentration by lower intensity, less sharply defined edges, and by the fact that when a solution of oxyhemoglobin is treated with a reducing agent—e. g., hydrazin hydrate—the characteristic single broad band of hemoglobin appears, while on treating a solution of NO-hemoglobin with the same reagent, no reduction takes place and the bands are not affected.

NO-hemoglobin is practically unaffected on treatment with potassium ferricyanid in neutral solution, with Stokes's solution (ammoniacal ferro-tartrate), with sodium nitrite, or with hydrazin hydrate, but is gradually reduced by potassium ferricyanid in acid solution.

When a solution of NO-hemoglobin is treated with ether in the presence of a small quantity of alcohol or of dilute acid, a bright-red colored extract is obtained which shows a distinct absorption band just at the right of the D line, and occasionally, in concentrated solution, a faint band at the left of the E line. In the absence of acid or alcohol no color is extracted by ether. In a previous paper the writer considered that the color extracted under the above conditions was NO-hemoglobin, but from work which he has done since that time it is evident that the color extracted by ether is a derivative of NO-hemoglobin, produced apparently by the reducing action of the alcohol or acid upon the NO-

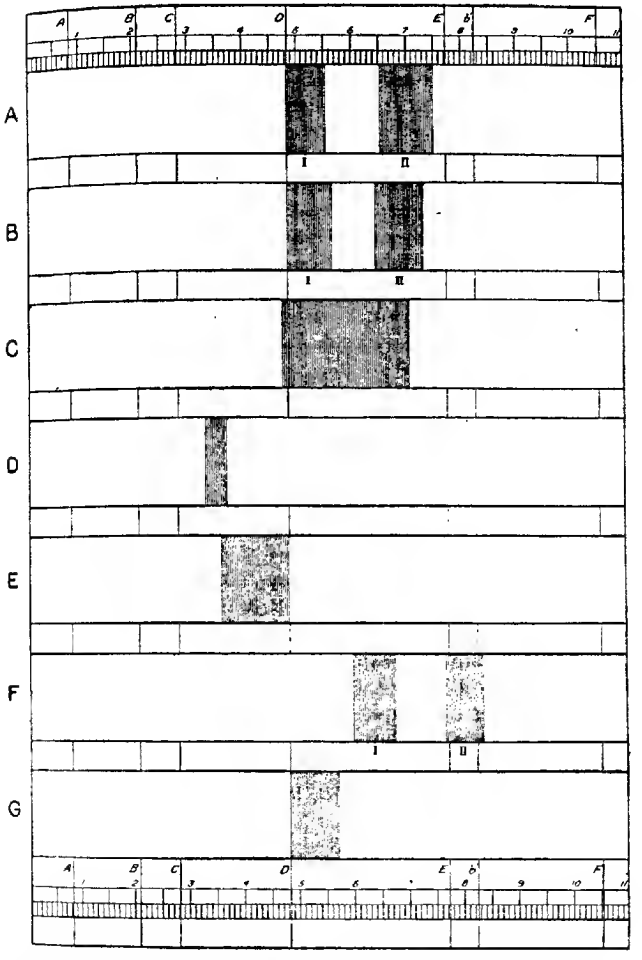

FIG. 1.—Spectra of hemoglobin and some of its derivatives. *A*, absorption spectrum of a solution of oxyhemoglobin; *B*, absorption spectrum of a solution of NO-hemoglobin; *C*, absorption spectrum of a solution of hemoglobin prepared by treating a solution of oxyhemoglobin with hydrazin hydrate; *D*, absorption spectrum of a solution of methemoglobin prepared by treating a solution of oxyhemoglobin with potassium ferricyanid; *E*, absorption spectrum of an alkaline solution of hematin; *F*, absorption spectrum of a solution of hemochromogen prepared by treating an alkaline solution of hematin with hydrazin hydrate; *G*, absorption spectrum of a solution of NO-hemochromogen.

hemoglobin. In pure solutions nitric oxid is insoluble in ether. The nature of this ether-soluble derivative of NO-hemoglobin will be discussed in connection with the color of cooked salted meats.

PREPARATION OF PURE NO-HEMOGLOBIN IN DRY CONDITION.—Twenty-five c. c. of a concentrated solution of NO-hemoglobin were cooled to 0° C., 6 c. c. of absolute alcohol previously cooled to the same temperature were added, and the dish was gently rotated. An abundant quantity of dark cherry-red crystals formed immediately. The dish was covered, placed in a refrigerated compartment for 24 hours at a temperature of −4° C., and the contents then filtered with the aid of suction in a room held at a temperature of +1° to +4° C. There was obtained a quantity of reddish brown crystals which were partly soluble in water, giving a reddish brown solution which showed a spectrum of NO-hemoglobin contaminated by the presence of methemoglobin. The material was not sufficiently soluble in water to allow of recrystallization.

A large number of trials were made with various methods of procedure in an endeavor to obtain pure NO-hemoglobin in dry condition, but without much success. Crystallization by a method using alcohol seems to change the NO-hemoglobin, in part at least, to methemoglobin.

When crystallization was carried on in the presence of a reducing agent—e. g., hydrazin hydrate or Stokes's solution—moist crystals could be obtained which showed a spectrum of NO-hemoglobin free from methemoglobin; but on drying the crystals in vacuo over sulphuric acid a change to methemoglobin took place.

CRYSTALLIZATION OF NO-HEMOGLOBIN.—In general, the method used by Reichert and Brown (1909) for the crystallization of hemoglobin and oxyhemoglobin was followed. In brief, the procedure was as follows: A pure concentrated solution of NO-hemoglobin was prepared by the methods previously described, and was examined spectroscopically to determine its freedom from other hemoglobin derivatives. Ammonium oxalate, in the proportion of 2 gm. to 100 c. c., was then added to the solution, which was then shaken to dissolve the salt. All subsequent procedure was carried on in a refrigerated room at a temperature of +1° to +5° C.

A few drops of the NO-hemoglobin solution were placed on a microscopic slide and allowed to evaporate until a heavy, dry proteid ring had formed around the drop. The cover glass was then carefully applied so as to exclude air bubbles, and the edges were sealed with balsam. Microscopic examination was made immediately after mounting and at intervals thereafter, according to the rate of crystal formation. The formation of crystals started at the dried proteid ring and proceeded toward the center of the mount, although occasionally crystals of ammonium oxalate would form, and the hemoglobin crystals would start from this base.

The above method was followed for the crystallization of both oxyhemoglobin and NO-hemoglobin from sheep, ox, and pig blood, and of methemoglobin from ox blood. It was found practically impossible to secure crystals of hemoglobin or of its derivatives from the blood of the above-mentioned animals without the use of ammonium oxalate. It was also found necessary to carry on the work at a temperature slightly above freezing, since the crystals would not form readily at room temperature.

OX-BLOOD OXYHEMOGLOBIN.—Plate XXXII, figures 1 and 2, shows crystals of oxyhemoglobin from ox blood. These crystals correspond very closely with those described by Reichert and Brown. No attempt was made to make a critical study of the crystallography of any of the various hemoglobin compounds studied.

OX-BLOOD NO-HEMOGLOBIN.—Plate XXXII, figure 3, shows crystals of this compound. It was found very difficult to obtain crystals of NO-hemoglobin, owing, apparently, to its greater solubility as compared with oxyhemoglobin. The crystalline structure, it will be noted, is distinctly different from that of oxyhemoglobin.

OX-BLOOD METHEMOGLOBIN.—Plate XXXII, figures 4 and 5, shows crystals of this compound which were prepared by treating oxyhemoglobin with potassium ferricyanid. It will be noted that the crystals of this compound are very similar in structure to those of NO-hemoglobin.

SHEEP-BLOOD OXYHEMOGLOBIN.—Plate XXXIII, figures 1 and 2, shows crystals of this compound which correspond very closely with those described by Reichert and Brown.

SHEEP-BLOOD NO-HEMOGLOBIN.—It was not found possible to secure a thoroughly satisfactory mount of these crystals, owing to their high solubility. Plate XXXIII, figure 3, shows fair crystals of this compound in the form of plates, which, however, are largely obscured by the large crystals of ammonium oxalate. It may be noted, however, that the crystals of NO-hemoglobin are distinctly different from those of oxyhemoglobin from the same source.

PIG-BLOOD NO-HEMOGLOBIN.—Plate XXXIII, figures 4 and 5, shows crystals of NO-hemoglobin obtained from pig's blood. It was not found possible to secure a good mount of oxyhemoglobin crystals from pig's blood, but the NO-hemoglobin crystals shown in this illustration are very different in structure from the oxyhemoglobin crystals from pig's blood described by Reichert and Brown.

The above work shows that NO-hemoglobin derived from ox, sheep, and pig blood is a crystallizable compound, with a definite structure, depending upon the species from which it has been obtained, and that its structure is different from oxyhemoglobin crystals from the same sources.

COLOR OF UNCOOKED SALTED MEATS

To a sample of finely ground fresh beef was added 0.2 per cent of potassium nitrate, and the material was placed in a refrigerated room at a temperature of 34° F. for seven days. At the end of that period the meat had a bright-red color, but gave evidences of incipient putrefaction. On extraction with 95 per cent alcohol, a faintly red-colored extract was obtained, which on spectroscopic examination showed a faint band just at the right of the D line. The color of the extract quickly faded. On extraction with water the sample yielded a dark-red extract, which retained its color without change for several days. On spectroscopic examination the following spectrum was observed: Just at the right of the D line a very heavy dark band, and a trifle to the left of the E line a heavy band, but wider than and not quite so dark as the first band and with less sharply defined edges. These bands correspond exactly in position with those of oxyhemoglobin and NO-hemoglobin, but more closely resemble the latter.

On treatment with sodium-nitrite solution the extract changed somewhat in color to a reddish brown, but still showed the two absorption bands noted above, and in addition showed a methemoglobin band at the left of the D line. The presence of this band would indicate that the extract contained some oxyhemoglobin, which on treatment with sodium nitrite changed to methemoglobin. On treatment with hydrazin hydrate the extract was not changed in color, and showed the following spectrum: Just at the right of the D line a very heavy band; at the left of the E line a somewhat lighter and wider band with less sharply defined edges. This spectrum corresponds exactly with that of the original extract.

The position of the absorption bands just noted, the fact that these bands were unaffected on treatment of the extract with hydrazin hydrate, and the solubility of the color in water are sufficient proof that the color of the meat under examination was due to the presence of NO-hemoglobin.

The production of NO-hemoglobin in the sample of meat under examination is easily explained. The potassium nitrate added to the meat had been reduced, either by bacterial or enzym action or by both, to potassium nitrite, nitrous acid, and nitric oxid, with the consequent formation of NO-hemoglobin.

Samples of various uncooked meats and sausages in which saltpeter had been used as one of the curative agents were obtained on the market for the purpose of studying the coloring matter of these products, and the following results were obtained:

SMOKED PORK SHOULDER.—The freshly cut surface of the meat was bright red in color. On extraction with water the finely ground lean meat gave a dull-red colored extract, which showed the following spectrum: Just at the right of the D line a fairly heavy dark band. No other

bands were visible. On treatment with potassium ferricyanid the red color of the solution changed to brown and the band disappeared. On extraction with 95 per cent alcohol a red-colored extract was obtained which showed the following spectrum: Just at the right of the D line a fairly heavy band, in the same position as noted with the water extract. On treatment with potassium ferricyanid the color of the extract changed to brown, and the absorption band disappeared. The addition of sodium nitrite or of hydrazin chlorid caused practically no change in the color or spectrum of the extract.

SMOKED PORK HAM.—The cut surface had a bright-red color. On extraction of the finely-ground lean meat with water a red-colored extract was obtained which showed the following spectrum: A fairly heavy band just at the right of the D line. The addition of sodium nitrite or of hydrazin hydrochlorid caused no change in color of the extract.

SALAMI SAUSAGE.—This product is known as a dry summer sausage, and is cured by hanging in a dry and well ventilated room for some time; it is also subjected to a light smoking. The cut surface of the meat had a bright-red color. On extraction with alcohol a bright-red colored extract was obtained which showed the following spectrum: A heavy dark band just at the right of the D line. On treatment with hydrazin chlorid the solution became somewhat milky in appearance, but the color and spectrum were not affected. Treatment with sodium nitrite caused no change in the color or spectrum of the extract, while potassium ferricyanid changed the red color of the solution to brown, and the absorption band disappeared.

DRIED BEEF.—This product is first cured in a brine containing salt, sugar, and saltpeter, and is then smoked. The cut surface of the meat was dark red. On extraction with alcohol a bright-red colored extract was obtained, which showed the following spectrum: A heavy dark band just at the right of the D line. Treatment with reducing agents gave the following results: Potassium ferricyanid destroyed the red color, and the absorption band practically disappeared; sodium nitrite partially destroyed the red color, but the absorption band at the right of the D line was still visible; hydrazin hydrate did not affect the color or spectrum of the extract. On extraction with water a faintly red-colored solution was obtained. The meat residue after extraction had a bright-red color.

SMOKED CERVELAT SAUSAGE.—This product is prepared in much the same manner as salami sausage. On extraction with alcohol a light-red colored extract was obtained which gave an absorption spectrum showing a distinct band just at the right of the D line. Treatment with hydrazin hydrate did not affect the color or spectrum of the solution, while sodium nitrite, hydrazin chlorid, and potassium ferricyanid destroyed the red color of the extract, and the absorption band disappeared.

Treatment of the sausage with water extracted no color, while ether gave a light-red colored extract, the color of which faded rapidly. Spectroscopic examination showed a distinct band just at the right of the D line.

CURED PORK SHOULDER, NOT SMOKED.—The freshly cut surface of the meat had a bright-red color. On treatment with water a light-red colored extract was obtained which showed the following spectrum: A distinct band just at the right of the D line. The color of the extract faded rapidly. On extraction with alcohol the meat gave a red-colored extract, showing a spectrum with a distinct band just at the right of the D line. Treatment with sodium nitrite or with hydrazin hydrate caused practically no change in the color or spectrum of the extract.

CORNED BEEF.—On extraction with water a rather cloudy, red-colored extract was obtained which gave a spectrum showing a fairly distinct band just at the right of the D line. Treatment with sodium nitrite caused no change in the color or spectrum of the solution. On extraction with alcohol the meat gave a bright-red colored extract which showed a fairly heavy absorption band just at the right of the D line. Treatment of the extract with sodium nitrite did not affect the color or spectrum, while hydrazin hydrate, hydrazin chlorid, and potassium ferricyanid destroyed the red color and the absorption bands of the solution.

SUMMARY OF RESULTS WITH UNCOOKED SALTED MEATS

The results of the examination of the samples of uncooked salted meats reported above may be summarized as follows:

The color of the meats was generally soluble in alcohol and in some cases in water. All samples gave extracts which showed an absorption band just at the right of the D line. In general, treatment with hydrazin hydrate or with sodium nitrite did not affect the color or spectrum of the extract. Potassium ferricyanid and hydrazin chlorid generally destroyed the red color of the extract and caused the absorption band to disappear. The fact that all samples of meats examined gave a red-colored extract with alcohol while only a part of them yielded a red-colored extract with water may be explained in this way: NO-hemoglobin is readily soluble in water, but insoluble in alcohol or ether. However, if a little alcohol is first added to a solution of NO-hemoglobin—sufficient to cause a slight precipitation of the proteid—and the solution is then extracted with ether, a bright-red colored extract may be obtained which gives a spectrum showing an absorption band just at the right of the D line. The coloring matter extracted by ether is undoubtedly a decomposition product of NO-hemoglobin.

In the case of the meats examined it would appear that all samples giving with water a red-colored extract that showed an absorption band

at the right of the D line had as their coloring matter NO-hemoglobin. In the case of those samples giving a red-colored extract with alcohol and showing an absorption band at the right of the D line but giving no red-colored extract with water, the coloring matter of the meat was not NO-hemoglobin, but a derivative of that compound. In the case of those samples which gave red-colored extracts with both alcohol and water and which showed an absorption band just at the right of the D line, the color is certainly due, in part at least, to NO-hemoglobin, and it may be due, in part, to a derivative of NO-hemoglobin present as such in the meat, or the alcohol may break down the NO-hemoglobin during extraction.

The evidence is ample to show that the action of saltpeter in the curing of meats is primarily to cause the formation of NO-hemoglobin; but it is very possible that under certain conditions of manufacture or processing to which salted meats are subject, the NO-hemoglobin may undergo changes.

RED COLOR OF COOKED SALTED MEATS

Haldane has shown that the red color of cooked salted meats is due to the presence of NO-hemochromogen, a reduction product of NO-hemoglobin to which the color of uncooked salted meats is due. NO-hemochromogen is characterized by its solubility in alcohol, its resistance to the action of reducing agents, and by a spectrum showing a distinct band just at the right of the D line and a faint band a trifle to the left of the E line. While Haldane's work seems to show clearly that the color of cooked salted meats is due to NO-hemochromogen, it has seemed desirable to study the subject further and to determine especially if the NO-hemoglobin of uncooked meats be reduced to NO-hemochromogen under other conditions than by cooking. The fact that in the examination of certain uncooked salted meats a coloring matter had been obtained similar to NO-hemoglobin yet not possessing all of the properties of that compound, as has already been noted, led the writer to believe that the coloring matter of some uncooked salted meats might be due, in part at least, to NO-hemochromogen.

NO-hemochromogen is but briefly mentioned in the literature. The compound is described by Linossier (1887), Haldane (1901), and by Abderhalden (1911).

The following experiments in the production of this compound were conducted by the writer. A dilute alcoholic, ammoniacal solution of hematin was saturated, first with hydrogen to remove air, then with nitric oxid, and finally again with hydrogen. On treatment with nitric oxid the brown color of the solution gradually changed to reddish brown; the characteristic spectrum of alkaline hematin disappeared, and in its place appeared a spectrum showing a fairly heavy band just at the right

of the D line and corresponding to the spectrum of NO-hemochromogen, as described by Haldane, except that the second band at the left of the E line was not visible. The addition of Stokes's solution caused no change in the color or spectrum of the solution. On standing overnight the red color of the solution had changed to brown and the spectrum had changed to that of alkaline hematin.

A dilute alcoholic, ammoniacal solution of hematin was treated with a small quantity of a concentrated solution of hydrazin sulphate. The brown color of the hematin solution quickly changed to the bright pink of hemochromogen, with a corresponding change in spectrum. The hemochromogen solution was then saturated with hydrogen, nitric oxid, and hydrogen in turn. On saturation with nitric oxid the pink color of the solution quickly changed to cherry red, and spectroscopic examination showed a heavy dark band just at the right of the D line. No other bands were observed, even though the solution was examined in a deep absorption cell.

The two substances produced, either by saturation of a solution of hematin with nitric oxid or by similar treatment of a solution of hemochromogen, are apparently identical. The evidence seems to show that this substance is the compound NO-hemochromogen.

The structural relation between NO-hemoglobin and NO-hemochromogen is simple. NO-hemoglobin is a molecular combination of nitric oxid and hemoglobin—the latter compound consisting of the proteid group, globin, on one hand, and the coloring group, hemochromogen, on the other. NO-hemoglobin and NO-hemochromogen differ from each other simply in that one contains the proteid group, globin, while the other does not. Apparently, then, a method of treatment which would split off the globin group from NO-hemoglobin should result in the production of NO-hemochromogen, provided, of course, that the procedure did not in turn change or destroy the NO-hemochromogen produced.

As has already been noted by Haldane, it was found that when a solution of NO-hemoglobin was heated to boiling a brick-red precipitate formed, in contrast to the dark-brown precipitate which formed on heating a solution of oxyhemoglobin or of blood. The brick-red precipitate was filtered off and was then extracted with alcohol, which gave a light-red colored extract showing a spectrum with a fairly heavy band just at the right of the D line. This spectrum corresponds with that of NO-hemochromogen. On standing, the color of the extract faded rapidly.

A solution of oxyhemoglobin was treated with one-hundredth normal nitrous acid and showed a spectrum corresponding to that of NO-hemoglobin. The color of the solution changed from the bright red of oxyhemoglobin to the dark cherry-red characteristic of NO-hemoglobin. Treatment with sodium nitrite did not affect the color or spectrum of the

solution. The production of NO-hemoglobin by this method is undoubtedly due to the action of the nitric oxid, liberated from the unstable nitrous acid, upon the hemoglobin. On heating the solution to boiling a brick-red precipitate was formed which, after filtration and on extraction with alcohol, gave a light-red colored extract which showed a spectrum with a fairly heavy band just at the right of the D line. Treatment with sodium nitrite did not affect the color or spectrum of the solution.

A piece of lean beef about 3 inches square and 1¼ inches thick was placed in a cold 0.1 per cent solution of sodium nitrite, and the solution was brought to the boiling point and boiling continued for one-half hour. Immediately on placing in the cold nitrite solution the surface of the meat turned dark brown in color; but on boiling, the meat took on a bright-red color, similar to that of cooked corned beef. On cutting the cooked meat the cut surface showed a bright-red color extending from the surface for about one-fourth of an inch toward the center of the piece, the interior of the meat being dark brown. The meat was ground and extracted with alcohol, which gave a bright-red colored extract showing a spectrum with a quite heavy band at the right of the D line and a very faint band at the left of the E line.

The color which Lehmann obtained on extracting with alcohol meat which had been cooked in water containing nitrites and acid, he named "hæmorrhodin." Results obtained by Haldane and by the writer seem to show, however, that the compound described by Lehmann is none other than NO-hemochromogen.

The following kinds of salted meats were used in studying the color of cooked salted meats: Salami, cervelat, Frankfurter, and Bologna sausages, corned beef, cured pork shoulder (unsmoked), smoked shoulder, and smoked ham. Bologna and Frankfurter sausages are cooked in the process of manufacture, so that these products are cooked salted meats. Portions of each of the samples of meats were cooked for some time in boiling water, then finely ground and extracted with alcohol. In all cases a red-colored extract was obtained, the intensity of the color varying with the product. On spectroscopic examination the extract from each sample showed a distinct band just at the right of the D line, but in practically all cases a second absorption band was not visible. The addition of sodium nitrite, as a rule, did not affect the color or spectrum of the extract. The color of the cooked salted meats was soluble in ether, but insoluble in water.

The evidence seems to show very clearly that the color of cooked salted meats is due to the NO-hemochromogen resulting from the reduction of the NO-hemoglobin of the raw salted meats on boiling.

It was found that the alcoholic extracts from the same product, whether in raw or cooked condition, showed practically identical prop-

erties. This does not signify that the coloring matter of raw salted meat is necessarily NO-hemochromogen, but rather it appears that treatment with alcohol reduces the coloring matter of uncooked salted meats, NO-hemoglobin, to NO-hemochromogen. In the case of certain uncooked salted meats NO-hemochromogen may be present as such before extraction with alcohol. The best means of differentiating between NO-hemoglobin and NO-hemochromogen as the coloring matters of salted meats seems to be on the basis of their differences in solubility. NO-hemoglobin is soluble in water but insoluble in ether and alcohol, while NO-hemochromogen is soluble in ether and alcohol and insoluble in water. In other respects the properties of the two compounds are very similar.

In the case of certain kinds of uncooked salted meats and meat food products—e. g., summer sausage and dried beef—the coloring matter seems to be NO-hemochromogen rather than NO-hemoglobin. The color is insoluble in water, but is soluble in alcohol and exhibits other properties of NO-hemochromogen.

It is very probable that in the case of meats which have been cured with saltpeter or of meat food products in which saltpeter has been used in the process of manufacture, the reduction of NO-hemoglobin to NO-hemochromogen takes place to a greater or lesser degree, depending upon conditions of manufacture and storage. The two compounds are so closely allied that their differentiation in one and the same product is not a matter of great importance.

CONCLUSIONS

The results of the investigation reported in this paper may be briefly summarized as follows:

The color of uncooked salted meats cured with potassium nitrate, or saltpeter, is generally due, in large part at least, to the presence of NO-hemoglobin, although the color of certain kinds of such meats may be due in part or in whole to NO-hemochromogen.

The NO-hemoglobin is produced by the action of the nitric oxid resulting from the reduction of the saltpeter used in salting upon the hemoglobin of the meat.

The color of cooked salted meats cured with saltpeter is due to the presence of NO-hemochromogen resulting from the reduction of the color of the raw salted meat on cooking.

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PLATE XXXII

Fig. 1.—Oxyhemoglobin, ox blood.

Fig. 2.—Oxyhemoglobin, ox blood.

Fig. 3.—NO-hemoglobin, ox blood.

Fig. 4.—Methemoglobin, ox blood. Prepared by treating oxyhemoglobin with potassium ferricyanid.

Fig. 5.—Methemoglobin, ox blood. Prepared by treating oxyhemoglobin with potassium ferricyanid.

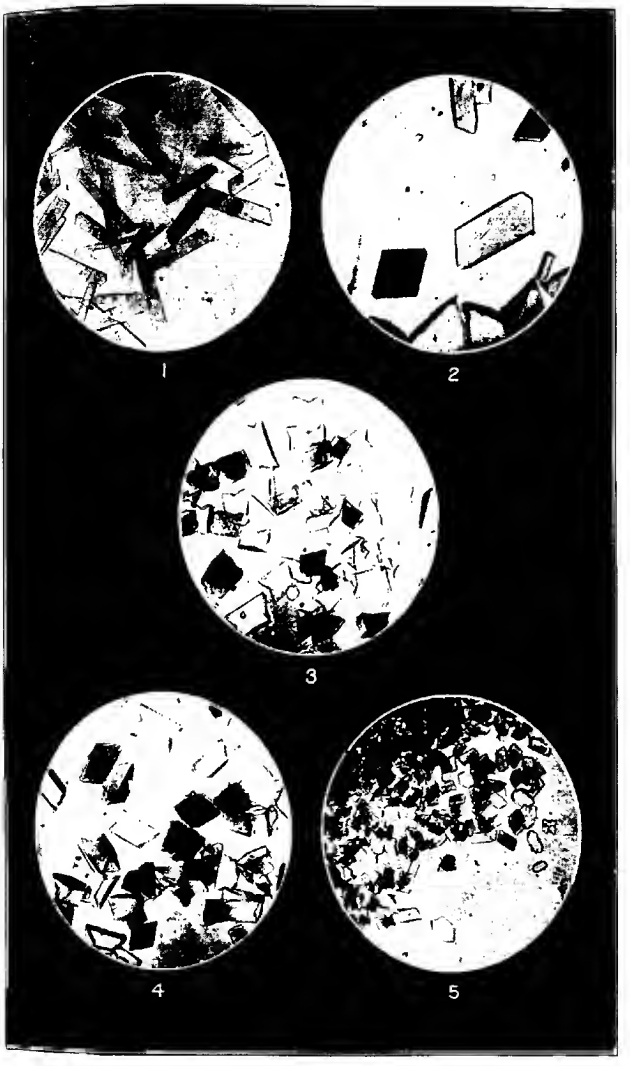
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PLATE XXXIII

Fig. 1.—Oxyhemoglobin, sheep blood.
Fig. 2.—Oxyhemoglobin, sheep blood.
Fig. 3.—NO-hemoglobin, sheep blood.
Fig. 4.—NO-hemoglobin, pig blood.
Fig. 5.—NO-hemoglobin, pig blood.

OIL CONTENT OF SEEDS AS AFFECTED BY THE NUTRITION OF THE PLANT

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INTRODUCTION

Although oils and fats are very widely distributed in the plant world, the commercial supply of these products is derived chiefly from a comparatively small number of species, and in most cases the seeds of these plants furnish the raw material. In general, the oil produced by the plant is usually stored in the seed or other reproductive parts, and for this and other reasons the seed constitutes the most favorable material for a study of the quantitative production of oil in plants.

The seed as a rule varies less in composition than other plant parts, as would be inferred when we consider its relatively small size along with the fact that it normally possesses the ability to reproduce in detail the distinctive characters of the parental type. Nevertheless, when grown under widely different conditions, seeds frequently show such marked changes in composition that their agricultural or commercial value is materially affected. The composition of certain seeds, more particularly wheat and other grains, as influenced by environment, has been extensively investigated in so far as relates to their content of protein, carbohydrate, and ash; but no extensive investigation of the oil content of seeds as affected by the various factors of nutrition has thus far been reported. There are on record, however, numerous analyses of oleaginous seeds grown in different regions, which indicate marked differences in oil content, presumably due, at least in part, to the varying conditions under which the seeds were produced. Some of our important crop plants are of value, primarily, for the oil contained in the seed; and it is a matter of practical importance to ascertain, so far as possible, the most favorable conditions for obtaining maximum yields of oil. It was with this object in view that an investigation was undertaken, some of the results of which are presented in the present paper.

The true fatty oils, composed of glycerin esters of the fatty acids, are quite different chemically from the carbohydrates; and, in fact, the two groups of compounds have little in common, except that both contain only the three elements—carbon, hydrogen, and oxygen. On the other hand, there is a very intimate and significant physiological rela-

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C

Vol. III, No. 3
Dec. 15, 1914
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tionship between the carbohydrates and the oils in the seed and other parts of the plant. Outside of the living cell the transformation of carbohydrate into fat, or the reverse process, has not been accomplished; but both of these processes are readily carried out by the living protoplasm. The researches of Müntz (1886),[1] Leclerc du Sablon (1895, 1896), Gerber (1897 a, b), Ivanow (1912), and others with the poppy (*Papaver somniferum*), flax (*Linum usitatissimum*), rape (*Brassica* spp.), soy bean (*Glycine hispida*), castor-oil plant (*Ricinus communis*), walnut (*Juglans regia*), sweet almond (*Amygdalus communis*), hemp (*Cannabis sativa*), and sunflower (*Helianthus annuus*) all go to show that the development of oleaginous seeds is characterized by a progressive accumulation of oil accompanied by a corresponding decrease in carbohydrates. Under proper conditions this transformation takes place in unripe seeds detached from the mother plant, further indicating that the oil is derived from the carbohydrate. Although oleaginous seeds in general are relatively rich in protein and the accumulation of oil proceeds simultaneously with that of protein, no evidence exists that there is any direct relationship between the two processes.

From the researches of the investigators mentioned, together with those of Schulze (1910) and his pupils, it may be inferred that the plant during the period prior to blooming normally accumulates an adequate supply of nutrients, chiefly in the form of carbohydrate and protein, to insure the development of the seed. At or near the blooming stage there begins a general movement of nutrients in the form of the simpler sugars and soluble nitrogenous constituents through the stem toward the reproductive parts. During the first stages of development of the seed the carbohydrates are laid down largely in the form of cellulose and hemicellulose, in order to provide for the early development of the testa, or seed coat, which serves first as conductive tissue for the embryo and in later stages as a protective membrane but not as a depository of surplus food. Then follows in the seed of some species a marked accumulation of so-called reserve carbohydrates, mainly starch and hemicellulose, while in other species the nitrogen-free "reserve" food accumulates in the form of oil. Examination of the stem parts during the development of the seed has shown that the organic non-nitrogenous nutrients flowing into oleaginous seeds are largely the same as for starchy seeds—namely, soluble carbohydrates.

While there appears to be no doubt that the oil in the plant cell, at least in the higher plants, is derived from carbohydrate, the mechanism of the reactions involved is not understood, since the transformation is not known outside the living cell.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 249.

PROBLEMS INVOLVED AND GENERAL METHODS OF PROCEDURE

The growth, development, and composition of the individual plant depend on the combined action of heredity and environment. It follows that in any investigation of heredity or of environment in relation to plant life such methods should be followed as will make it possible to distinguish between these two forces. This principle of clearly distinguishing between the effects of environment and those due to heredity is simple enough in theory, but in practice it is often extremely difficult to follow because of interrelations between the two forces which are not fully understood. Thus, nonheritable variations in the progeny of relatively pure strains grown under what would seem to be uniform environmental conditions become especially apparent when we come to deal with quantitative differences such as are involved in a study of variable chemical composition as influenced by the factors of nutrition. In our experiments on the nicotine content of tobacco, the oil content of seeds of various species, and other quantitative characters, efforts to insure the greatest uniformity possible in environmental conditions have failed to effect anything like uniform composition in the progeny of individuals representing the purest strains available. Some of the strains of tobacco under study had been inbred for eight generations.

It follows that in dealing with quantitative differences in composition as influenced by environment a sufficiently large number of individuals must be used to avoid misleading results due to variations that can not be brought under control. Where conditions have made it impracticable to grow relatively large numbers of plants for study, the alternative of repeating the experiment has been adopted. It is equally important to use caution in reaching generalizations based on results obtained with an insufficient number of species or varieties. In the course of our work on the oil content of seeds it has been frequently observed that in the case of the soy bean, for example, different varieties are not always influenced in the same manner by the environment. In such plants as the soy bean and cotton, therefore, as many varieties as practicable have been included in the experiments. In selecting plants for study those that are of special importance by reason of the oil content of the seeds and which are otherwise adapted to the work in view have been taken. Thus far we have utilized cotton (*Gossypium* spp.), soy bean, peanut (*Arachis hypogaea*), and sunflower.

With reference to chemical composition, the commercial value of seeds evidently depends on the relative percentages of their several constituents, but from the standpoint of the grower the returns also depend on the quantity of seed produced—that is, on the number and size of the seeds produced by the individual plant or on a given acreage. Hence, in the

present investigation the weight of the seed in addition to its percentage composition was determined in order to ascertain the actual quantity of oil which it contained. When practicable, data were collected as to the size and character of growth of the plant as a whole for purposes of correlation and more particularly in dealing with specific factors of the nutrition process as a measure of the relative effects produced by the special conditions of the experiment. The actual yield of seed has not received any special attention, since this is greatly affected by factors of nutrition which have little influence on the problems involved, and, moreover, the matter of crop yields constitutes a separate problem. In analyzing the seed the official method[1] for the determination of oil was followed, using anhydrous ether as the solvent.

OIL CONTENT OF SEED AT SUCCESSIVE STAGES OF DEVELOPMENT

It has been shown that the accumulation of oil in the seed does not set in actively during the very earliest stages in the development of the seed, and the work of Ivanow (1912) suggests that there is a period of very intense oil formation, which occurs about midway between blooming and the final maturity of the seed. This raises the question as to the existence of a "critical period" in oil formation which would have an important bearing on the effects of external conditions on the quantity of oil produced. Samples of soy beans and of cotton seed were collected at different stages of maturity, to secure more definite information on this point. The weight of the seed also was obtained in each case, so as to ascertain the changes in the absolute as well as the relative oil content. To arrest respiration promptly and thereby avoid changes in composition, the immature seeds were dried in the oven at 70° to 80° C. In the case of the soy bean 5 to 6 pods were picked from each of 100 to 125 plants in taking the samples. The material was grown at the Arlington Experiment Farm, Va., the Peking soy bean in 1910 and the others in 1912. The several pickings from each variety were taken from the same plants at stated intervals, but for obvious reasons the different pickings do not necessarily represent the actual growth made by the beans in the intervals covered. They are strictly comparable, however, as to the relation between the oil content and the size of the seed at the several stages of maturity. In the case of the cotton seed care was taken to obtain the same number of immature and mature bolls from each plant, and these were always taken from the same branch, about 12 plants being used for each pair of samples. The results obtained are shown in Tables I and II.

[1] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 11 fig. 1908.

TABLE I.—*Oil content of soy beans gathered at various stages of maturity at Arlington Experiment Farm, Va., in 1910 and 1912*

VARIETY, S. P. I. NO. 19981

Date harvested.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.	Date harvested.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
	Gm.	*Per cent.*	*Per cent.*	*Gm.*		*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
Aug. 13	3.53	7.50	2.95	0.104	Sept. 11	356.8	6.60	18.05	64.4
20	39.3	5.50	10.65	4.19	16	447.0	7.55	18.02	80.5
23	96.24	5.80	13.40	12.9	21	472.3	5.95	18.60	87.8
28	146.2	6.10	15.94	23.3	25	498.7	7.44	18.37	91.6
Sept. 3	263.7	5.40	16.70	44.0	30	487.0	6.35	18.85	90.8
6	282.0	6.55	17.00	47.9	Oct. 7	479.3	7.55	17.77	85.2

VARIETY, S. P. I. NO. 21755

July 24	8.2	7.00	3.80	0.312	Aug. 13	158.0	5.80	14.75	23.3
30	32.3	5.50	9.38	3.03	20	217.4	6.00	15.83	34.4
Aug. 3	81.8	6.30	11.58	9.48	26	210.5	5.00	15.65	32.9
7	98.3	5.40	12.52	12.3					

VARIETY, S. P. I. NO. 32907

Aug. 20	3.28	6.70	3.38	0.110	Sept. 4	67.3	5.85	16.95	11.4
23	12.5	6.50	7.65	.955	7	72.1	5.70	17.20	12.4
27	23.2	5.15	12.10	2.81	11	85.0	6.00	18.10	15.4
30	49.2	5.15	14.95	7.36	20	82.5	5.55	18.28	15.1

TABLE II.—*Oil content of immature and mature cotton seed grown at Thompsons Mills, Ga., in 1910*

Variety and condition of seed.	Lint.	Weight of 1,000 seeds.	Hulls.	Meats.	Moisture in meats.	Oil in moist meats.	Oil in whole seed.	Oil in 1,000 seeds.
Toole.	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*
Immature	41.8	78.4	44.6	55.4	3.80	37.65	20.9	16.4
Mature	38.1	93.3	38.8	61.2	4.05	38.03	23.3	21.7
Trice:								
Immature	31.8	102.0	45.6	54.4	3.72	34.60	18.8	19.2
Mature	30.8	134.4	42.4	57.6	4.00	36.17	20.9	28.2
Sam McCall:								
Immature	35.3	133.0	40.4	59.6	3.95	35.80	21.4	28.5
Mature	33.5	155.6	39.3	60.7	4.05	37.93	22.9	35.7

In all cases the relative weight of the seed rather than the date of harvesting is to be taken as the more nearly correct index of the stage of development. The first pickings of the soy beans were made when the seeds were exceedingly small, and the final pickings represent the fully matured seed. The results are definite and conclusive. Except for the period immediately following blooming and that directly preceding final

maturity, there is throughout the development of the seed a gradual and rather uniform gain in the oil content as compared with the growth of the seed. There is no evidence of a definite "critical period" for the accumulation of oil during the development of the seed. Considering only the percentage of oil, there is a very sharp increase during the first few weeks after blooming, and then only a slow gain until near the end of the ripening. During the final stage of ripening there is a decrease both in the size of the seed and in the oil content. This phenomenon, which was observed also by Müntz (1886), is probably due to continued respiratory activity after assimilation has ceased. In the case of the cotton seed the immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots. As in soy beans, the increase in oil proceeds somewhat more rapidly than the growth of the seed.

OIL CONTENT OF SEED AS AFFECTED BY RATIO OF LEAF SURFACE TO QUANTITY OF SEED PRODUCED

It has been shown that the accumulation of oil in the seed proceeds throughout the greater portion of the period of development and ripening. It also has been pointed out that during the so-called vegetative period preceding blooming there is an accumulation of carbohydrates in the leaves and stems which is later utilized in the formation of the oil deposited in the seed. From these facts it might be inferred that the total quantity of oil stored in the seed would be affected by the relative extent of the photosynthesizing plant parts, more particularly the leaves, while, on the same basis, the percentage content of oil might be influenced by the ratio between the photosynthesizing parts and the quantity of seed produced. In other words, it is to be expected that premature shedding of leaves, such as often happens under adverse conditions, or the shedding of a portion of the blossoms would affect the accumulation of oil in the seed. As regards the quantity of seed produced, a diminished supply of accumulated carbohydrate might lead to the production of a smaller number of seeds or of smaller sized seeds, or possibly both.

For the reasons indicated it is apparent that in any analytic study of oil production in the seed as related to factors of nutrition account must be taken of the possible effects of the nutrition conditions during the two principal life periods of the plant—namely, the vegetative and the reproductive. The production of oil by the plant requires favorable conditions for the accumulation of carbohydrate during the vegetative period and for the transformation of carbohydrate into oil during the second period, although there may be, of course, more or less overlapping of the two processes. As a special phase of the influence of the accumulation of carbohydrate on oil formation, experiments were carried out with soy beans in which the normal distribution of the vegetative and reproductive plant parts was modified by partial defoliation and by removal of a portion of the blossoms or very young seed pods. The number of plants used in each

experiment ranged from 35 to 50. Data were also obtained as to the effects of the two treatments on the general development of the plant, as indicated by the height, the weight of the air-dry root and stalk minus the leaves, and the total yield of seed. The results are presented in Tables III and IV.

TABLE III.—*Oil content of soy beans as affected by partial defoliation*

Variety and treatment.	Date of blooming.	Average weight of stalk and root.	Average height of plant.	Average yield of beans per plant.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
		Gm.	*Inches.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
S. P. I. No. 32907:								
Control	July 30	33.7	22.2	56.6	89.8	7.45	16.75	15.0
Number of leaves reduced to about 40 per cent of normal on June 29, July 16, 22, 30, and Aug. 14	do	20.7	19.1	34.1	85.4	6.65	17.52	15.0
Number of leaves reduced to about 50 per cent of normal at same periods as above	do	24.0	20.4	42.2	85.6	7.40	17.80	15.2
Control	do	36.8	25.2	51.2	87.1	6.60	17.20	15.0
Number of leaves reduced to about 40 per cent of normal on Aug. 1 and 15	do	24.1	21.5	30.7	81.8	6.75	17.85	14.6
S. P. I. No. 21755:								
Control	July 8	9.7	11.1	24.5	209.0	8.65	16.21	33.9
Number of leaves reduced to about 40 per cent of normal on July 15 and 30	do	5.3	9.7	11.5	168.5	8.55	16.32	27.5
S. P. I. No. 30599:								
Control	do	24.5	27.0	61.1	179.4	6.20	19.95	35.8
Number of leaves reduced to about 50 per cent of normal on July 15 and 22	do	16.5	21.5	53.2	167.8	6.25	20.93	35.1
S. P. I. No. 30745:								
Control	do	24.5	24.1	58.6	192.6	7.95	19.80	38.1
Number of leaves reduced to about 40 per cent of normal on July 15 and 22	do	10.7	19.2	32.1	180.0	6.05	20.35	36.6
S. P. I. No. 30593:								
Control	do	27.2	24.4	59.4	190.3	6.70	20.93	39.8
Number of leaves reduced to about 50 per cent of normal on July 15 and 75 per cent Aug. 12	do	13.8	19.9	33.8	169.4	6.40	21.30	34.2

Considering, first, the effects of partial defoliation (Table III), in all cases the weight of the root and stalk, the height of the plant, and the total yield of beans are decidedly reduced. The size of the beans, however, is only slightly reduced, so that the decreased yield is due almost entirely to the smaller number of beans developed. It is an interesting fact that the small decrease in size of the beans is almost exactly offset by the increase in percentage of oil, so that the actual quantity of oil in the individual seed remains practically the same. This fact only holds apparently within certain limits, for in the case of the variety designated as "S. P. I. No. 30593," where the defoliation was nearly three-fourths complete, the decrease in size of the bean was too great to be fully offset by the higher percentage of oil. The most striking exception, however, is shown by S. P. I. No. 21755. This variety differs from the others in that the seed are matured a very short time after blooming. In the present case the seed were fully ripe on August 26, whereas

all of the remaining varieties were about five weeks later in reaching full maturity. It appears, therefore, that a degree of defoliation which but slightly modified the size and oil content of the seed in those varieties requiring a long period for the development of the seed brought about a much more decided effect in the variety which is able to fully develop rather large-sized seed in a very short period of time. Another reason for the greater effect of the defoliation on the size of the bean, as well as on the total yield of beans, in S. P. I. No. 21755 is that the foliage normally is less abundant than that of the other varieties.

TABLE IV.—*Oil content of soy beans as affected by partial removal of very young seed pods*

Variety and treatment.	Date of blooming.	Average weight of stalk and root.	Average height of plant.	Average yield of beans per plant.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
		Gm.	*Inches.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
S. P. I. No. 32907:								
Control	July 30..	36.3	25.7	50.2	84.1	6.45	17.15	14.4
Large number of pods removed on Aug. 19	...do....	49.7	27.2	55.0	103.1	6.65	17.69	18.2
S. P. I. No. 21755:								
Control	July 8...	9.7	11.1	24.5	209.0	8.65	16.21	33.9
Larger portion of pods removed on July 15 and 22	...do....	9.0	10.8	9.8	204.6	8.00	15.83	32.4
S. P. I. No. 30599:								
Control	...do...	24.5	27.0	61.1	179.4	6.20	19.95	35.8
Larger portion of pods removed on July 15 and 22	...do....	36.2	21.5	52.6	202.2	6.40	20.02	40.4
S. P. I. No. 30745:								
Control	...do....	24.5	24.1	58.6	192.6	7.95	19.80	38.1
Larger portion of pods removed on July 15 and 20 and Aug. 19	... do....	38.7	24.2	39.1	227.8	6.35	19.15	43.7
S. P. I. No. 30593:								
Control	...do....	27.2	24.4	59.4	190.3	6.70	20.93	39.8
More than two-thirds of pods removed on July 15 and 20 and Aug. 12	...do....	42.1	23.3	25.7	244.4	6.50	19.95	48.8

It might be expected that the effects produced by removing a portion of the young seed pods would be largely the reverse of those produced by partial defoliation, and this is found to be true in part. Removing a portion of the pods resulted in much heavier root and stalk. The effect on yield of the reduction in the number of beans allowed to develop is offset to a considerable extent by a notable increase in the size of the beans. The increase in the size of the bean is not associated with a corresponding decrease in the percentage of oil; hence, the actual quantity of oil in the individual seed is considerably increased. Here, again, the early-maturing variety, S. P. I. No. 21755, stands out as an exception. Reducing the number of seed allowed to develop failed to increase the weight of the vegetative parts or the size of the seed and its oil content.

Considering the two sets of experiments together, when the development of the seed extends through a relatively long period, a reduction of, say, 50 per cent in the normal proportion of leaves or photosynthetic organs leads to a decreased weight of the other vegetative parts, as well as of the total yield of seed, but the size of the seed is only slightly re-

duced, and the quantity of oil in the individual seed is scarcely changed. A reduction in the normal proportion of the reproductive parts, on the other hand, leads to an increase in weight of the vegetative parts, and the size of the seed and its oil content are materially increased. These facts are not applicable to the variety which developed its seed within a short period of time.

OIL CONTENT AS AFFECTED BY SIZE OF SEED

In studying quantitative relationships of seeds one is at once confronted with the fact that in any particular lot of seed there is always considerable variation in the size of individuals, whatever the conditions under which the seed may be grown. This is true even of the seed from an individual plant. To ascertain whether there is any constant relationship between the oil content and the size of the seeds from the same plant, seeds of several varieties of soy beans grown in different localities were separated by hand into the larger and smaller sizes and their oil content was determined. The results are shown in Table V.

TABLE V.—*Oil content of soy beans of large and small size from the same plant*

Variety and locality.	Size of beans.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.
		Gm.	*Per cent.*	*Per cent.*
Ogemaw (S. P. I. No. 17258):				
Pullman, Wash.	Large	171	5.60	13.88
Do.	Small	106	5.25	14.17
Amherst, Mass.	Large	290	6.30	16.92
Do.	Small	169	6.60	16.63
Wooster, Ohio.	Large	259	6.40	16.30
Do.	Small	137	6.40	15.95
Hansen (S. P. I. No. 20409):				
Pullman, Wash.	Large	40	6.10	11.72
Do.	Small	25	5.85	12.27
Statesville, N. C.	Large	64	7.35	13.27
Do.	Small	37	6.80	13.50
La Fayette, Ind.	Large	86	6.75	11.72
Do.	Small	53	6.75	11.15
Buckshot (S. P. I. No. 17251):				
Kingston, R. I.	Large	364	6.30	17.45
Do.	Small	196	6.65	17.00
Amherst, Mass.	Large	389	6.65	17.87
Do.	Small	215	6.40	16.95

Considering only the large and small beans from the same lot of seed, it appears that generally the percentage of oil is approximately the same, although there are some cases in which there are considerable differences. No fixed rule can be laid down as to the relative percentages of oil in large and small beans. It is evident that there are marked differences in the size of the beans in each lot, but a quantitative separation is impracticable, since there are all gradations in size. The only practicable method of comparing different lots of seed, therefore, is to secure average values based on comparatively large quantities, simply counting out the seed as they come, without any attempt at separation into sizes.

OIL CONTENT AS AFFECTED BY LENGTH OF GROWING PERIOD

Independently of any differences between early and late varieties as such, it might be expected that difference in oil content would be influenced by both the character and the length of the growing period. Several investigators have concluded that the length of the growing period is an important factor in determining the starch and protein content of wheat. In plants of determinate growth, in which all the seed are developed during approximately the same period, the effect of the length of the growing period on the oil content may be studied by making successive plantings at given intervals, since many of such species show a marked tendency to shorten the growing period when planted abnormally late. Mooers (1909) has called attention to this tendency in soy beans, and the present authors have determined the oil content in the seed of several varieties of this crop planted at stated intervals during the spring and summer months.[1] The results of the tests are shown in part in Table VI.

TABLE VI.—*Oil content of soy beans planted at intervals of two weeks in 1911*

Variety and date of planting.	Number of days from planting to blooming.	Number of days from blooming to full maturity.	Number of days from planting to full maturity.	Weight of 1,000 beans.	Moisture in beans.	Oil in beans.	Oil in 1,000 beans.
				Gm.	*Per cent.*	*Per cent.*	*Gm.*
S. P. I. No. 21755:							
May 1	40	62	102	186	6.90	17.25	32.4
May 15	35	58	93	153	7.05	16.38	25.1
June 1	37	50	87	166	6.90	17.40	28.9
June 15	32	54	86	145	7.10	17.27	25.0
July 1	27	58	85	168	7.60	15.51	26.1
Haberlandt (S. P. I. No. 17271):							
May 1	61	83	144	210	6.65	19.73	41.4
May 15	54	76	130	211	6.70	19.47	41.1
June 1	50	74	124	217	6.35	20.32	44.1
June 15	45	74	119	240	6.80	18.95	45.5
July 1	44	72	116	211	7.20	17.35	42.3
Buckshot (S. P. I. No. 17251):							
May 1	40	72	112	271	6.40	19.15	51.9
May 15	40	69	109	272	6.40	19.62	53.4
June 1	39	68	107	277	6.20	19.35	53.6
June 15	39	58	97	250	6.10	18.95	47.4
July 1	33	64	97	291	6.40	18.43	54.2
Medium Yellow (S. P. I. No. 17269):							
May 1	56	102	158	329	6.45	17.60	57.9
May 15	49	94	143	313	6.25	18.25	57.1
June 1	50	77	127	312	5.70	17.75	55.4
June 15	45	73	118	305	5.80	17.38	53.0
July 1	41	66	107	334	6.30	16.43	54.9

[1] In these experiments the authors are indebted to the Office of Forage-Crop Investigations, of the Bureau of Plant Industry, for samples of the soy beans which were grown at the Arlington Experiment Farm, Va., together with the data as to time of planting and date of maturity.

The different plantings of soy beans show marked variations in the size of the beans and in their oil content, but there is no definite relationship between these characters and the date of planting. In other words, the character rather than the length of the season in which the seed is developed seems to be the important factor. The seed from the latest plantings contain a somewhat lower percentage of oil than the others, but this relationship is not verified in other tests which have been omitted from Table VI for the sake of brevity. These additional data all show lack of definite relationship between size and oil content of the seed and the length of the growing period. There is a remarkable difference between varieties as to the shortening of the period required for maturing seed when planted late, as illustrated by the Buckshot and the Medium Yellow varieties.

VARIETAL DIFFERENCES IN OIL CONTENT OF THE SEED

As a preliminary to the study of oil content as affected by nutrition, it was necessary to ascertain the relation of heredity to the quantity of oil produced in the seed in so far as relates to the comparative behavior of different varieties. This work has been limited largely to soy beans and cotton, since collections of varieties of the other oil-producing plants investigated have not been available.

The method of procedure has been to grow a number of varieties of each species under uniform conditions, to use the purest seed obtainable, and to repeat the tests for several seasons, using the precautions which have been discussed above in drawing samples for analysis. In Table VII are given the results obtained from material furnished by the Office of Forage-Crop Investigations, representing seven varieties of soy beans grown at the Arlington Experiment Farm in 1907, 1908, and 1910.

TABLE VII.—*Varietal differences in the oil content of soy beans grown at Arlington Experiment Farm, Va., in 1907, 1908, and 1910.*

Variety and year grown.	Weight of 1,000 beans.	Moisture in beans	Oil in moist beans.	Oil in 1,000 beans.
Shanghai (S. P. I. No. 14952):	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
1907	215.4	6.65	19.55	42.1
1908	186.1	6.16	18.37	34.2
1910	217.1	6.80	20.20	43.8
Average	206.2	6.54	19.37	40.0
Eda (S. P. I. No. 17257):				
1907	280.6	6.30	19.70	55.5
1908	263.4	6.17	19.90	52.5
1910	269.4	6.15	21.55	58.2
Average	271.1	6.21	20.38	54.7

TABLE VII.—*Varietal differences in the oil content of soy beans grown at Arlington Experiment Farm, Va., in 1907, 1908, and 1910*—Continued

Variety and year grown.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.
Yosho (S. P. I. No. 17262):	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
1907	182. 2	6. 90	16. 78	30. 6
1908	170. 2	6. 35	16. 25	27. 7
1910	202. 8	6. 65	15. 44	31. 3
Average	185. 0	6. 63	16. 16	29. 9
Amherst (S. P. I. No. 17275):				
1907	193. 6	6. 65	20. 15	39. 1
1908	179. 3	5. 50	19. 50	34. 9
1910	176. 0	6. 05	20. 33	35. 7
Average	182. 9	6. 07	20. 00	36. 6
S. P. I. No. 19981:				
1907	335. 8	6. 60	20. 67	69. 6
1908	311. 4	4. 85	20. 20	62. 8
1910	331. 9	6. 45	21. 86	72. 8
Average	326. 0	5. 97	20. 91	68. 4
S. P. I. No. 20410:				
1907	56. 9	6. 80	15. 67	8. 9
1908	63. 4	6. 18	15. 55	9. 9
1910	64. 6	6. 40	15. 45	10. 0
Average	61. 6	6. 46	15. 56	9. 6
S. P. I. No. 22312:				
1907	224. 2	7. 30	17. 72	39. 5
1908	185. 3	4. 93	16. 00	29. 6
1910	214. 1	6. 65	18. 90	40. 4
Average	207. 9	6. 29	17. 54	36. 5

In Table VIII are shown the results from six varieties of Upland cotton, also a foreign species (Hawasaki) grown in the Piedmont section of Georgia and the Coastal Plain region of South Carolina. The data presented are the averages for the years 1909, 1910, and 1911 in these two sections, and in each case all plantings were from the same original lot of seed.

TABLE VIII.—*Varietal differences in the oil content of cotton seed grown in northern Georgia and in the Coastal Plain region of South Carolina*

[Average for three years]

Variety of cotton.	Cotton seed grown in—											
	Northern Georgia.						Coastal Plain region of South Carolina.					
	Lint.	Weight of 1,000 seeds.	Hulls.	Moisture in kernels.	Oil in moist kernels.	Oil in 1,000 seeds.	Lint.	Weight of 1,000 seeds.	Hulls.	Moisture in kernels.	Oil in moist kernels.	Oil in 1,000 seeds
	Per cent.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*
King	36.8	103.8	40.5	4.98	36.54	22.6	36.7	96.9	41.8	4.79	39.14	21.7
Russell	31.5	154.5	42.4	5.13	37.40	32.9	33.2	139.2	44.3	4.89	40.73	31.3
Shine	32.7	108.2	41.3	4.93	37.52	23.4	34.1	95.7	44.1	4.54	41.23	21.8
Toole	36.8	93.1	39.7	4.98	37.33	20.7	38.5	85.2	42.1	4.66	40.87	20.1
Dixie	32.9	111.0	40.6	4.71	38.06	25.2	33.7	100.4	42.7	4.69	42.07	24.8
Hawkins	35.0	110.8	42.2	4.76	36.72	23.7	34.2	99.0	46.4	4.56	40.83	21.0
Average	34.3	113.6	41.1	4.92	37.26	24.8	34.9	102.8	44.6	4.69	40.81	23.5
Hawasaki	41.1	73.1	51.4	6.01	30.73	10.9	41.0	63.0	52.1	4.93	32.25	9.8

The data in Table VII show that there are enormous varietal differences in soy beans both as to size of seed and as to oil content. Furthermore, it should be noted that the seasonal effects of the three years did not influence the several varieties alike with respect to either of these two characters. More extensive tests through a period of five years and with several additional varieties fully confirm these results. It is clear, therefore, that in soy beans heredity is a very important factor, not only with respect to the size and the oil content of the seed but also as regards the extent to which these characters respond to change in environment. The results with cotton, as shown in Table VIII, are quite different. There are marked varietal differences in size of seed and other important characters, but the percentage of oil is remarkably constant when the environmental conditions are the same. Williams (1906) obtained somewhat greater variations in oil content in a test with 21 varieties.

EXTENT TO WHICH THE ENVIRONMENT MAY AFFECT THE OIL CONTENT

Before entering upon a study of the individual factors of nutrition in their relation to the formation of oil in the plant, it was desired to obtain some idea as to the extent to which the quantity of oil accumulating in the seed may be influenced by change in the general environment. Some varieties of soy beans may be grown under a very wide range of conditions, and for this reason this plant was largely used in the experiments. Through the cooperation of the Office of Forage-Crop Investigations several varieties of soy beans were grown by a number of the State experiment stations, and samples of the seed were subjected to analysis. The results of the experiments are given in Table IX.

TABLE IX.—*Oil content of soy beans grown under different environmental conditions*

Variety and locality.	Weight of 1,000 beans.	Moisture in kernels.	Oil in kernels.	Oil in 1,000 beans.
Hansen (S. P. I. No. 20409):	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*
Wooster, Ohio	50.1	5.15	11.90	6.0
Statesville, N. C.	51.0	5.10	12.95	6.6
Pullman, Wash.	39.4	5.95	12.25	4.8
La Fayette, Ind.	72.7	5.15	11.05	8.0
Auburn, Ala.	50.9	6.40	12.38	6.3
Kingston, R. I.	64.5	6.05	12.00	7.7
Buckshot (S. P. I. No. 17251):				
Wooster, Ohio	251.2	5.85	16.40	41.2
Pullman, Wash.	156.3	5.80	14.55	22.7
La Fayette, Ind.	334.6	6.10	13.25	44.3
Auburn, Ala.	250.4	5.75	20.60	51.6
Kingston, R. I.	347.5	5.70	18.00	62.6
Guelph (S. P. I. No. 17261):				
Wooster, Ohio	164.0	5.82	16.20	26.6
Statesville, N. C.	269.0	6.12	20.05	53.9
La Fayette, Ind.	190.9	5.32	18.40	35.1
Auburn, Ala.	182.4	5.90	20.00	38.1
Kingston, R. I.	196.1	5.00	17.65	34.6
Ogemaw (S. P. I. No. 17258):				
Wooster, Ohio	207.4	5.70	16.45	34.1
Statesville, N. C.	193.5	5.15	17.05	33.0
Pullman, Wash.	137.8	5.50	14.40	19.8
La Fayette, Ind.	249.6	6.00	13.70	34.3
Auburn, Ala.	233.3	5.80	19.25	44.9
Kingston, R. I.	235.6	6.05	17.00	40.0

In some cases there are differences of more than 100 per cent in the size of the seed, and also very large differences in the percentage of oil, when soy beans are grown in different localities. It is evident that environment, as well as heredity, may affect tremendously the size of the seed and the quantity of oil stored therein. It should be noted again, however, that the behavior of the four varieties of soy beans was by no means the same when grown in different localities. There seems to be one exception to this observation—namely, that the conditions at Pullman, Wash., were such as to produce in each case abnormally small seed.

As cotton does not thrive in a cool climate, it has not been practicable to study the development of oil in the seed under such a wide range of conditions as in the case of soy beans. It can not be stated, therefore, whether the quantity of oil stored in the seed is subject to as wide fluctuations as have been noted in soy beans. By reference to Table VIII it will be seen that in a 3-year test all of the six varieties of cotton produced considerably heavier seed, containing a decidedly lower percentage of oil, when grown in the Piedmont section of northern Georgia than when grown in the Coastal Plain region of South Carolina. The increase in size of seed in northern Georgia was not entirely offset by the decrease in percentage of oil, so that the

actual quantity of oil stored in the seed produced under these conditions was somewhat greater than in those grown in the Coastal Plain region. There was also considerable yearly fluctuation in the oil content of the seed in both sections, owing to varying seasonal conditions. (See Table X.) As already noted, the uniformity in behavior of all the varieties of cotton contrasts sharply with the varietal differences observed in soy beans. The average difference in oil content for the six varieties of cotton as grown in the two localities is greater than the varietal differences in either locality.

OIL CONTENT OF SEED AS AFFECTED BY SOIL

In dealing with the response of the plant to differences in the environment, it is frequently sought to differentiate between the effects of climate and those ascribed to the soil. Climate, or the average weather, as ordinarily understood, refers to conditions of the atmosphere, of which temperature and moisture are perhaps the most important as factors of nutrition. But the soil is likewise subject to variation in temperature and moisture, and there must be a tendency toward equilibrium in temperature and moisture between these two media in which the plant lives.

It is true that under any fixed weather, or climatic, conditions plants grown on contrasted soil types may show well-defined differences in their development, but such relationships are subject to change with any change in the climatic factors. There is ample evidence to show that the differences in plant development observed on contrasted soil types during one season may be completely reversed in another season. Again, it is true that in extreme cases differences in climate may produce certain definite differences in plant development more or less independently of the soil type. Within ordinary ranges of soil and climatic differences, however, it is hardly possible to develop far-reaching generalizations as to the specific effects of either independently of the other, for change of climate results in a change of soil conditions, and vice versa.

In spite of the above-mentioned limitations, which must apply in considering soil and climate as environmental factors, it seemed desirable to obtain data as to the influence of differences in soil type on the accumulation of oil in the seed as produced under varying seasonal conditions. In the experiments with cotton six representative Upland varieties were grown for three consecutive years (1909–1911) at Thompsons Mills, Ga., on two adjoining but contrasted types of soil, the original lots of seed being used for each year's planting. For convenience these soil types are designated as "red soil" and "gray soil," respectively. Both belong to the Cecil series and were in a good state of cultivation. The red soil is a comparatively heavy, tenacious clay, while the gray soil is an open-textured sandy loam. As all of the varieties were affected in a

similar manner, the results of the experiments as given in Table X are averages for the six varieties.

TABLE X.—*Average oil content of six varieties of cotton seed grown for three seasons on two different soil types at Thompsons Mills, Ga.*

Year in which grown.	Lint.		Weight of 1,000 seeds.		Hulls of seed.		Moisture in kernels.		Oil in moist kernels.		Oil in 1,000 seeds.	
	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.	Red soil.	Gray soil.
	Per cent.	*Per cent.*	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Gm.*	*Gm.*
1909	34.2	35.3	114.2	109.7	41.3	41.7	4.15	4.12	37.69	36.46	24.2	22.5
1910	32.6	33.6	121.5	115.9	40.3	41.2	4.08	4.02	36.67	38.53	26.8	26.4
1911	35.2	36.0	110.0	107.4	40.8	41.4	6.50	6.44	37.41	37.33	24.4	23.7
Average	34.0	35.0	115.2	111.0	40.8	41.4	4.91	4.86	37.28	37.44	25.1	24.0

Taking the average results for the three years, the red soil gave only slightly heavier seed, with a somewhat smaller proportion of hulls than the gray soil, and there was practically no difference in the oil content. Each year the seed was heavier and contained a smaller proportion of hulls on the red soil than on the light soil, but the case is quite different as regards the oil content. In 1909 the oil content was considerably higher on the red soil than on the gray, while in 1910 these relations were reversed and in 1911 the differences practically disappeared. In other words, the comparative effects of the two soil types depend on the seasonal conditions, and it so happened that there was a balancing effect for the three years covered by the experiment.

Extensive data have been accumulated as to the effect of soil type on the oil content of soy beans, but these data are too voluminous to present in detail, and only a summary of the results can be given here. In 1911 plantings of six varieties of soy beans were made on the two above-mentioned soil types at Thompsons Mills, Ga., and data were secured as to the relative oil content of the beans. The data given in Table XI are the averages of three plantings, made on May 6, June 3, and June 21, respectively, for the six varieties designated as S. P. I. Nos. 17254, 17263, 17857, 18227, 19984, and 20892. In 1912 five varieties were grown on a heavy clay soil at the Arlington Experiment Farm and on an infertile sandy soil containing a large percentage of coarse sand. The latter soil is composed largely of material dredged from the Potomac River and is designated as Potomac Flats soil. These two soils are only about half a mile apart, so that the weather conditions during the growing season were essentially the same. While, as stated, five varieties were used in this experiment, data for only two of these, S. P. I. Nos. 30599 and 30745, as a basis for comparison with the greenhouse experiment next described, are given in Table XI.

In addition to these field experiments with the soy bean, a number of tests on different soil types were made under specially controlled conditions. In the spring of 1912 the two varieties designated as S. P. I. Nos. 30599 and 30745 were grown on the above-mentioned heavy clay and Potomac Flats soils placed in the greenhouse at the Arlington Experiment Farm. The two soils occupied adjoining portions of the same bench, received the same quantities of water, and were exposed to the same conditions of light, etc. In 1911 and 1912 soy beans were grown in different soil types contained in large earthen pots set into the soil. These pots consisted of glazed tiles 3 feet long and 18 inches in diameter. A perforated bottom of concrete was set in each tile cylinder 3 inches from the lower end. The tiles were then set into the soil so that the upper ends extended 3 inches above the ground level. The cylinders were filled to the ground level with the different soils. Series of tiles were thus installed in the clay soil of the Arlington Farm and in the Norfolk fine sandy loam in the vicinity of Manning, S. C. At each location one-half the total number of cylinders were filled with the native soil and the second half with soil transported from the other point. In preparing the soils for the tiles the surface soil and the subsoil were collected separately and each lot very thoroughly mixed. In each case a sufficient quantity of the native soil thus prepared was shipped in bags to the other point, so that presumably at each point there was a series of insulated cores of two very different soil types embedded in the native soil. Apparently the insulation from the surrounding soil should be practically perfect, except possibly as regards temperature, and a special experiment, in which a cylinder was inclosed in a second one of much larger size and filled with the same soil, indicated that the temperature of the surrounding soil had but little effect on that contained in the cylinder. The two soil types, of course, would be exposed to exactly the same weather conditions in any particular locality. A second series of cylinders was installed at the Arlington Farm and filled with the native clay soil and with Hartford fine sandy loam from South Windsor, Conn. In 1912 an additional set of cylinders, filled with the Potomac Flats soil, was used in connection with the first series.

In the first series of cylinders the variety designated as S. P. I. No. 17852B was used in 1911, while S. P. I. No. 32907 was used in 1912. In the second series S. P. I. No. 21755 was used. The results of the various tests with soy beans are summarized in Table XI. Each test is complete in itself and of course can not be compared directly with the others, since, with one exception, different varieties were used and the climatic conditions were not the same. The results of each separate test are based on data from a considerable number of individuals of each variety, usually 50 to 100, and in most cases are the averages from several varie-

ties. In all cases where two or more varieties are averaged the direction of the soil effect was the same for each of the varieties, though the extent of this effect was not the same.

TABLE XI.—*Oil content and related data of soy beans and peanuts grown on different soil types*

Locality, conditions under which grown, and kind of soil.	Soy beans.						Peanuts.			
	Dry weight of 100 stalks and roots.	Yield of beans from 100 plants.	Weight of 1,000 beans.	Moisture in beans.	Oil in moist beans.	Oil in 1,000 beans.	Weight of 1,000 seed.	Moisture in seed.	Oil in moist seed	Oil in 1,000 seed.
Thompsons Mills, Ga.:										
Field experiment—	*Gm.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*Gm.*
Gray land	.....	.....	144.4	4.41	20.44	29.5	553.1	3.07	47.25	261.6
Red land	.....	.....	136.3	4.20	21.68	29.5	540.7	2.80	50.70	274.3
Arlington, Va.:										
Field experiment—										
Arlington clay	2,250.0	5,911.0	180.0	7.20	19.85	36.0	356.0	4.40	47.85	170.4
Potomac Flats	301.0	636.0	130.9	8.02	21.00	27.5	411.0	3.55	47.34	168.6
Greenhouse experiment—										
Arlington clay	325.0	856.0	184.1	6.02	19.88	36.6	.....	.....	.....	...
Potomac Flats sand	351.0	561.0	169.3	6.10	19.52	33.0	.....	.....	.....	....
Pot experiments, Series I, 1911—										
Arlington clay	.....	54.5	55.0	4.90	17.44	9.6	470.0	3.12	51.68	242.5
Norfolk fine sandy loam	.....	70.8	65.3	5.10	16.62	10.8	521.0	2.35	49.16	254.4
Pot experiments, Series I, 1912—										
Arlington clay	908.0	687.0	68.1	7.02	18.55	12.7	478.0	3.90	50.25	240.4
Norfolk fine sandy loam	810.0	562.0	89.6	7.02	15.32	13.7	509.0	3.45	46.07	234.6
Potomac Flats sand	689.0	416.0	88.7	7.36	14.89	13.2	473.0	4.15	46.12	118.1
Pot experiments, Series II, 1912—										
Arlington clay	188.0	250.0	132.6	7.62	16.57	22.0	.....	.....	.....	.....
Hartford fine sandy loam	326.0	482.6	145.1	7.36	15.22	22.1	.....	.....	.....	.....
Manning, S. C.:										
Pot experiments, Series I, 1911—										
Arlington clay	.....	52.6	95.1	5.10	16.76	16.0	465.5	3.43	49.91	233.0
Norfolk fine sandy loam	.....	64.8	87.7	5.05	16.50	14.5	489.5	3.47	49.80	239.5
Pot experiments, Series I, 1912—										
Arlington clay	.....	.....	91.1	6.45	16.69	15.7	357.4	3.66	47.62	170.1
Norfolk fine sandy loam	.....	.....	96.3	6.50	16.83	16.2	363.8	3.73	47.04	171.0

These tests include a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant but depend largely on seasonal conditions, as was brought out in the experiments with cotton. The results in the field experiment at Arlington Farm as compared with those obtained in the greenhouse with the same soils illustrate this point. In the field test the plants suffered considerably from drought during the growing season, and here the sandy soil gave decidedly smaller beans and higher relative oil content than the clay soil. In the greenhouse the difference in size of beans largely disappeared, while the clay loam gave a somewhat higher percentage of oil than the sandy soil. In the pot experiments at Arlington Farm the lighter soils gave somewhat larger seed with lower percentages of oil than the heavier clay soil, but at Manning, S. C., there were no significant differences.

Experiments similar to those with soy beans were made with the peanut of the variety known as Spanish, and the results are given in Table XI. The effects produced by the different soil types are of the same general character as with soy beans, although the behavior of the two species under similar conditions is not always the same. In 1913 a series of pot cultures with the sunflower were carried out at the Arlington Farm in the same manner as described for soy beans, using a number of different soils in the test. The weights in grams per 1,000 seeds as grown in the Arlington clay, Norfolk sandy loam, Potomac Flats soil, Norfolk sand, and the so-called Benning sand were 90.5, 73, 79.5, 56.4, and 52, respectively, while the corresponding percentages of oil in the kernels were 51.25, 50.30, 55.70, 51.26, and 49.10. In this case soil differences brought about very marked differences in size of the seed, but the variations in relative oil content were less decided.

OIL CONTENT OF SEED AS AFFECTED BY CLIMATE

On comparing the data given in Table IX with those in Table XI it becomes apparent that the variations in the size of seed and the oil content of soy beans attributable to differences in soil type are far less than those observed when both soil and climate differ. The same relationships are observed in cotton seed, as shown in Tables VIII and X. These results are interpreted as indicating that under practical conditions climate is a more potent factor than the soil in modifying the size of seed and its oil content. The most probable explanation is that the atmosphere is subject to greater and more rapid variations in moisture and particularly in temperature, and also that the "soil climate" is greatly influenced by the weather conditions. Temperature and moisture differences of both soil and atmosphere are among the important factors of environment which may influence the plant characters under study, and this factor-complex must be at least partially analyzed before satisfactory conclusions can be reached as to the principal external factors concerned in oil formation in the plant.

OIL CONTENT OF SEED AS AFFECTED BY FERTILIZERS

The experiments with different soil types previously described have included soils varying greatly in fertility, as indicated by the comparative growth of the plants shown in Table XII, and the results as a whole show that within the limits ordinarily met with in farm practice the relative fertility of the soil does not very greatly influence the size of the seed or its oil content. A large number of fertilizer tests with cotton were carried out at Lamar and Timmonsville, S. C., in 1909, 1910, and 1911, to obtain more accurate information as to the effects of fertilizers on the size and oil content of the seed. The data are too voluminous to present in detail, but in Table XII a summary of the results for 1911

is given. In each series the results are averages of duplicate plots, except for the controls, which represent the averages for four plots in each case, all plots being one-fortieth of an acre in area. The tests in 1909 and 1910 included plots receiving four different quantities of nitrogen, four of phosphoric acid, and three of potash. Dried blood, acid phosphate, and muriate of potash were used as fertilizers.

TABLE XII.—*Results of tests with cotton at Manning, S. C., to determine the influence of fertilizers on the oil content of the seed*

Plot series No.	Plant food elements applied per acre.			Yield of seed cotton per acre.	Lint.	Weight of 1,000 seeds.	Hulls of seed.	Oil in kernels.
	Nitrogen.	Phosphoric acid.	Potash.					
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*
1	0	0	0	530	36.2	118	47.1	33.56
2	30	90	20	1,070	37.6	130	44.9	37.48
3	60	90	20	880	34.2	130	45.0	33.85
4	0	0	0	525	36.9	120	46.8	32.99
5	30	90	40	1,265	35.9	135	44.3	38.07
6	60	90	40	1,410	34.4	139	43.9	36.40
7	0	0	0	483	36.9	121	47.3	34.38
8	30	90	60	1,160	35.9	134	45.8	38.86
9	60	90	60	1,320	34.6	137	44.0	36.78
10	0	0	0	502	36.1	122	47.6	33.49
11	30	150	60	1,030	35.8	136	43.2	38.60
12	30	150	40	1,090	36.1	136	44.7	37.48
13	60	150	40	1,220	35.2	138	43.6	36.65

The soil used in 1911 was very poor, as shown by the large increases in crop yields produced by the complete fertilizers. The addition of all three elements, nitrogen, phosphorus, and potassium, combined in varying proportions, gave in all cases considerably heavier seeds, with a smaller percentage of hulls and a higher oil content in the kernels as compared with the controls. With respect to the varying quantities of the three fertilizer elements, increased applications of nitrogen had no appreciable effect on the weight of the seed and only a slight effect on the percentage of hulls, but lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium did not materially affect any of these characters. The tests of the two preceding years gave similar results.

Pot-culture tests were made in 1911 with the Peking variety of soy beans, using the tile cylinders, previously described, filled with the Arlington clay soil. In a series in which phosphorus and potassium in a fixed ratio were added in three different quantities, the yields of beans were greatly increased and the weight of the seed was not changed, while the oil content was increased about 20 per cent. With

the addition of phosphorus alone, very much the same results were obtained; but with the addition of potassium alone there was only a small increase in yield and practically no increase in oil content. In similar tests with Spanish peanuts phosphorus gave a large increase in yield and slightly increased the weight of the peas, but had no effect on the oil content. Potassium had practically no effect on the yield, the weight of seed, or the oil content.

SUMMARY

Experiments with soy beans have shown that, except for the period immediately following blooming and that directly preceding final maturity, there is a fairly uniform increase in oil content, both relative and absolute, throughout the development of the seed, and no evidence was found that there is a critical period of very intense oil formation at any stage of seed development. Tests with cotton likewise indicate that the increase in oil proceeds somewhat more rapidly than the increase in the weight of the seed.

As a consequence of the physiological relationship of oil to carbohydrate, it appears that maximum oil production in the plant requires conditions of nutrition favorable to the accumulation of carbohydrate during the vegetative period and to the transformation of carbohydrate into oil during the reproductive period. As a special phase of this relationship between carbohydrate supply and oil formation in soy beans, it was found that when the normal distribution of the vegetative and reproductive plant parts was modified by partial defoliation (50 to 60 per cent) the yield of beans was decidedly reduced, but the size of the beans and their oil content were only slightly affected, except in the case of an early-maturing variety. On the other hand, the removal of a portion of the blossoms or young pods caused a notable increase in the size of the beans allowed to develop, but did not materially affect the percentage oil content.

There is always lack of uniformity in the size of the seed from an individual plant; but it was found that there was no correlation between the size of the seed and the percentage content of oil.

Some varieties of soy beans show a marked tendency to shorten the time required for reaching maturity when planted late in the season, but no correlation was found between the date of planting and the size of the seed or their oil content. These properties appear to be influenced more by the character than by the length of the growing period.

Different varieties of soy beans grown under the same conditions showed marked differences in oil content and very great differences in size of the seed. Although different varieties of cotton showed decided differences in the size of the seed, there was very little difference

in the percentage oil content. The different varieties of soy beans did not respond alike to changes in seasonal conditions.

In tests with several varieties of soy beans grown under a very wide range of conditions there were found differences of more than 100 per cent in the size of the beans and very large differences in oil content. Here, again, the different varieties were not affected alike by changes in the environment. It was not practicable to grow cotton under such diverse conditions, but the difference in oil content of the seed as grown in the Coastal Plain and the Piedmont regions of the South was greater than the varietal differences when grown in the same environment. All varieties respond very much alike to changes in the environment.

Because of the interdependence of soil and climate with respect to temperature and water supply it is difficult or impossible to develop far-reaching generalizations as to the specific effects of either independently of the other on plant development. Six Upland varieties of cotton were grown three consecutive years on adjoining but contrasted soil types in northern Georgia. Each year the clay soil gave heavier seed than the sandy loam, but the relative oil content on the two soil types varied from year to year. In experiments with several varieties of soy beans only small differences were obtained in the size and oil content of the seed grown on these two soil types. Similar results were obtained with peanuts. Field experiments with soy beans and peanuts on sharply contrasted soil types at Arlington Experiment Farm, Va., and vicinity gave more decided differences in size and oil content of the seed. A number of tests with soy beans, peanuts, and sunflower were carried out also on different soil types under controlled conditions, using for the purpose large earthen pots set into the soil. The various tests were carried out under a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant, but depend largely on seasonal conditions.

From the data in hand it is concluded that under practical conditions climate is a more potent factor than soil type in controlling the size of the seed and its oil content, probably because those conditions of the atmosphere which constitute the climate largely control the corresponding conditions of the soil.

Within ordinary limits the relative fertility of the soil appears to be a minor factor in influencing the size of the seed and its oil content. In fertilizer tests with cotton the addition of a complete fertilizer to an unproductive soil gave larger seed and a considerably higher percentage of oil. Applications of nitrogen in increasing quantities did not affect the size of the seed, but lowered the percentage of oil, while increasing applications of phosphorus or potassium did not affect either character.

In pot-culture tests with soy beans the addition of phosphorus did not change the size of the seed, but increased the oil content. Potassium was without decided effect. In similar tests with peanuts neither phosphorus nor potassium affected the oil content.

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PRELIMINARY AND MINOR PAPERS

STUDIES IN THE EXPANSION OF MILK AND CREAM

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[A report on a series of experiments conducted for the Dairy Division, Bureau of Animal Industry]

INTRODUCTION

On May 27, 1913, the Dairy Division of the Bureau of Animal Industry requested the Bureau of Standards to determine the coefficient of expansion of market milk, single cream, and double cream. It was thought that the examination of a few samples of each would be sufficient to serve the purpose. Subsequent observations, however, showed that this was not the case. The wide variation of the rate of expansion of the samples first examined made it apparent that a much greater number of samples would be required than had been anticipated. The results here published are therefore the outgrowth of what was originally expected to be only a very few determinations.

OBJECT OF THE WORK

The principal object in undertaking the work was to determine the change in volume which occurs when the temperature of a given volume of milk or cream is changed and from the rate of change of volume to construct a table of relative volumes of milk and cream at various temperatures. For example, when milk is pasteurized and put into containers at a high temperature, it is sometimes desirable to know what volume of milk must be measured out at that temperature, in order that it may occupy a required volume at some standard temperature.

In the enforcement of the pure food and drugs laws by the Bureau of Chemistry it is held that a container shall have its full nominal capacity at 20° C. (68° F.)—that is, a container labeled as holding 1 gallon must hold 231 cubic inches at 20° C. For the sake of uniformity and also because 20° C. is a reasonable and convenient temperature, it has been chosen as the basis of the table of volumes of milk and cream published herewith. The samples of cream submitted by the Dairy Division were prepared from mixed herd milk produced at the Dairy Division Experiment Farm, Beltsville, Md. The percentage of fat was determined in each case by the Babcock test, and the samples are believed to be normal cream for the stated percentages of fat.[2]

METHOD OF DETERMINATION

The principle employed in determining the rate of expansion was to measure the change of density with change of temperature and from that to calculate the change in volume.

[1] The author would acknowledge his indebtedness to Miss Alice Purinton, formerly of the Bureau of Standards, and to Mr. E. L. Peffer for assistance rendered in the work.

[2] These samples were prepared and the fat determinations made by Mr. R. H. Shaw, of the Dairy Division.

The density determinations were made by what is commonly known as the method of hydrostatic weighing. By this method a sinker or plummet of known mass and volume is suspended in the liquid under examination and weighed. The density of the liquid is then calculated by means of the equation

$$D_t = \frac{W - w\left(1 - \frac{\rho}{8.4}\right)}{V_t}$$

in which D_t = density [1] of the liquid at the temperature t;
W = weight of sinker in vacuo;
w = apparent weight of sinker in liquid;
ρ = air density;
8.4 = assumed density of brass weights;
V_t = volume of sinker at temperature t

This method of determining densities, though very accurate when used under suitable conditions, is open to criticism when applied to a nonhomogeneous liquid, such as milk or cream. There is, of course, a constant tendency for the fat of the sample under investigation to separate out and rise to the surface and for the heavier components to sink to the bottom. The density of a nonhomogeneous liquid determined by this method will therefore tend to be too low if the sinker is suspended near the surface of the liquid and too high if suspended near the bottom. The difficulty, however, may be largely overcome by the frequent stirring of the sample and still more effectively by the use of a sinker of such a length as to reach nearly from the top to the bottom of the liquid. The average density of the displaced liquid will then be nearly the same as the average density of the whole mass of liquid, and the density determined will be nearly the average density of the sample.

DESCRIPTION OF APPARATUS

The apparatus employed in making the density determinations is described in publications of the Bureau of Standards.[2] Its essential parts are as follows:

The sample to be tested is placed in a tube surrounded by a water bath kept in constant circulation. This bath is in turn surrounded by another, which is also kept in constant circulation. The temperature of the outer bath may be kept constant or varied at will by the adjustment of the energy through an electric heating coil and by the flow of refrigerating brine in a coil, around which the water in the bath may be made to circulate. A sinker of known mass and volume is suspended from one arm of a sensitive balance placed above the other apparatus. The temperature is read from two mercury thermometers placed in water in a second tube similar to that in which the sample is placed, the two tubes being placed side by side within the inner circulating bath. The thermometers are suspended from a movable cover, which may be rotated to bring them successively into position for reading.

[1] Throughout this paper the term "density" is used to denote the mass per unit of volume, and is expressed in grams per milliliter. The densities are therefore numerically the same as specific gravities in terms of water at 4° C. as unity.

[2] Bearce, H. W. Density and thermal expansion of linseed oil and turpentine. U. S. Dept. Com. and Labor, Bur. Stand., Technol. Paper 9, 27 p., 1912.

Osborne, N. S., McKelvy, E. C., and Bearce, H. W. Density and thermal expansion of ethyl alcohol and of its mixtures with water. U. S. Dept. Com., Bur. Stand. Bul., v. 9, no. 3, p. 327-474, 12 fig., 1913.

METHOD OF PROCEDURE

The sample of milk to be investigated is placed in the containing tube, the sinker immersed, and the tube placed in position in the temperature-control bath. The temperature is then brought to the point at which the density is first to be measured and is allowed to remain constant until the apparatus reaches a condition of temperature equilibrium. Observations are then begun. First, a weighing is made with the sinker immersed in the sample and suspended from the arm of the balance. Then the temperature is read on each of the two thermometers; next, the sinker is detached and a weighing made with the sinker off, but with the suspension wire still passing through the surface of the liquid. The difference between these weighings is the apparent weight of the sinker in the liquid at the temperature of observation. The object of making the second weighing with the suspension wire still passing through the surface is to eliminate the surface-tension effect on the suspension wire. In order that the suspension wire may be kept straight and in position whether the sinker is attached or not, a small secondary sinker is kept suspended at all times, and the large sinker of known mass and volume is attached to that. When not attached, the large sinker rests on the bottom of the tube and remains standing in an upright position. Immediately after the weighing with the sinker detached, a second weighing is made with it again attached, after which the temperature is again observed on the two thermometers.

The observations at each point therefore consist of two weighings with the sinker attached, one weighing with it detached, and two readings on each of two thermometers. The reason for making two weighings with the sinker attached and only one with it detached is because in the former case a slight change of the temperature of the liquid will make an appreciable change in the weighing, on account of the large volume of the immersed sinker, while in the latter case the change is not appreciable.

After completing the observations at one point, the temperature is changed to the next one in the series and the process is repeated in the same order.

TEMPERATURE RANGE OF DENSITY DETERMINATIONS

At the beginning of the work it was intended to cover the temperature range from 0° to 50° C., but the rate of expansion at low temperatures was found to differ so much from the rate at higher temperatures that the expansion over the entire range could not be expressed by a simple equation. This is especially true of samples having a low fat content. In certain samples a point of maximum density was found at a temperature in the region of 5° C. This is what might be expected—from the similar behavior of water. In the samples containing higher percentages of fat the point of maximum density was not found, but the rate of expansion was noticeably less at the low than at the high temperatures.

The rate of expansion was especially desired at the higher temperatures, and since it was found that the results at temperatures between 20° and 50° C. could be well expressed by a simple equation, it was decided to cover only this temperature range, and when approximate results are desired beyond this range, to extrapolate from the results

over the range covered. The values at the lower temperatures obtained in this way will, of course, not be as near the truth as would be the case if density determinations were made at the low temperatures; but, on the other hand, to include determinations at the lower temperatures would render less accurate the reduced values at the higher temperatures—that is, the assumed equation would not come so near expressing the actual rate of expansion over the temperature range where accuracy is most desired. For that reason in making the "least squares" reduction of the observations at the various temperatures, only the observed densities at 20°, 30°, 40°, and 50° C. were considered.

CALCULATION OF RESULTS

After completing the observations given in Table I at as many points as desired, the density at each point was calculated, and from the density values at the different temperatures the rate of change was determined.

For convenience in calculation it was assumed that the rate of change of density with change of temperature could be expressed with sufficient exactness by means of an equation of the form

$$D_t = D_x + \alpha(t-x) + \beta(t-x)^2 + \gamma(t-x)^3 +$$

in which D_t = the density at any temperature t,

D_x = the density at some standard temperature x,

α, β, and γ = constants to be determined for each sample investigated.

In practice it was found that for certain temperature ranges the expansion was represented within the limits of experimental error, by the above equation, with all terms above the second power omitted. By means of a "least squares" method the observations on each sample given in Table I have been reduced and the calculated values of Dx, α, and β are given in Table IV. Observations and calculations of density for an average sample of cream are given in Table III. It will be seen from the closeness of the agreement between the calculated and the observed values of the density at various temperatures that the assumed equation comes very near expressing the actual rate of expansion of the different samples at the time the density determinations were made.

TABLE I.—*Observed densities of milk and cream*

Date.	Fat content.[a]	Temperature.	Density.	Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	°C.	*G. c. c.*	1913.	*Per cent.*	°C.	*G. c. c.*
July 12	0.025	0	1.0381	June 3	3.5	0	1.0362
	.025	10	1.0368		3.5	10	1.0348
	.025	20	1.0356		3.5	20	1.0320
	.025	30	1.0322		3.5	30	1.0284
	.025	40	1.0284				
	.025	50	1.0236				
July 11	2.5	0	1.0348	June 7	3.5	20	1.0311
	2.5	5	1.0343		3.5	30	1.0290
	2.5	10	1.0348		3.5	40	1.0237
	2.5	20	1.0317				
	2.5	30	1.0292	Nov. 19	3.5	20	1.0314
	2.5	40	1.0258		3.5	30	1.0279
	2.5	50	1.0212		3.5	40	1.0238
					3.5	50	1.0191

[a] The percentages of fat here given are as reported by the Bureau of Animal Industry at the time the samples were prepared.

TABLE I.—*Observed densities of milk and cream*—Continued

Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	*° C.*	*G./c. c.*
July 10.	5	0	1. 0344
	5	5	1. 0357
	5	10	1. 0353
	5	20	1. 0322
	5	30	1. 0299
	5	40	1. 0217
	5	50	1. 0171
Aug. 26.	5	20	1. 0268
	5	30	1. 0235
	5	40	1. 0192
	5	50	1. 0144
July 9.	7. 5	0	1. 0327
	7. 5	5	1. 0316
	7. 5	10	1. 0316
	7. 5	20	1. 0274
	7. 5	30	1. 0246
	7. 5	40	1. 0209
	7. 5	50	1. 0159
Nov. 19.	7. 5	20	1. 0261
	7. 5	30	1. 0226
	7. 5	40	1. 0184
	7. 5	50	1. 0136
July 8.	10	0	1. 0304
	10	5	1. 0310
	10	10	1. 0295
	10	20	1. 0242
	10	30	1. 0197
	10	40	1. 0152
	10	50	1. 0106
July 2.	15	0	1. 0256
	15	10	1. 0216
	15	20	1. 0170
	15	30	1. 0114
	15	40	1. 0061
	15	50	1. 0028
Aug. 25.	15	20	1. 0161
Aug. 26.	15	30	1. 0112
	15	40	1. 0061
	15	50	1. 0011
June 3.	20	0	1. 0242
	20	10	1. 0208
	20	20	1. 0137
June 7.	20	20	1. 0141
	20	30	1. 0081
	20	40	1. 0018
July 3.	20	0	1. 0214
	20	10	1. 0169
	20	20	1. 0106
	20	30	1. 0052
	20	40	. 9995
	20	50	. 9950

Date.	Fat content.	Temperature.	Density.
1913.	*Per cent.*	*° C.*	*G./c. c.*
June 27.	25	10	1. 0136
	25	20	1. 0070
	25	30	1. 0007
	25	40	. 9944
	25	50	. 9890
Nov. 20.	25	20	1. 0107
	25	30	1. 0028
	25	40	. 9965
	25	50	. 9911
June 26.	30	10	1. 0108
	30	20	. 9997
	30	30	. 9940
	30	40	. 9884
	30	50	. 9827
Aug. 23.	30	20	. 9966
	30	30	. 9918
	30	40	. 9864
	30	50	. 9813
Nov. 20.	30	20	. 9978
	30	30	. 9933
	30	40	. 9886
	30	50	. 9832
Nov. 21.	30	20	1. 0044
	30	30	. 9960
	30	40	. 9896
	30	50	. 9842
June 26.	35	0	1. 0129
	35	10	1. 0057
	35	20	. 9970
	35	30	. 9879
	35	40	. 9807
	35	50	. 9750
June 3.	40	0	1. 0088
	40	10	1. 0037
	40	20	. 9931
	40	30	. 9838
June 7.	40	20	. 9940
	40	30	. 9845
	40	40	. 9763
June 25.	40	0	1. 0050
	40	5	1. 0036
	40	10	1. 0027
	40	20	. 9931
Aug. 26.	40	30	. 9837
	40	40	. 9765
	40	50	. 9710

CALCULATION OF RELATIVE VOLUMES

After having determined the density of the several samples of milk and cream at the various temperatures, the observations for each sample were reduced by the method of least squares, as already stated, and the value of $D_{35°}$, α, and β determined for each sample. The values are shown in Table IV. The method of reducing the observations to obtain constants in the assumed equations—namely, values $D_{35°}$, α, and β—is as follows:

$D_t = D_{35°} + \alpha(t-35) + \beta(t-35)^2$.

$(C_1 = t - t_m;\ C_2 = C_1^2 - (C_1^2)_m;\ N = D_t - (D_t)_m.)$

$(\Sigma C_1^2\alpha + \Sigma C_{12}\beta C = \Sigma C_1 N.)$

$\Sigma C_1 C_2\alpha + \Sigma C_2^2\beta = \Sigma C_2 N.$

$500\alpha + 0 = -0.1990.$

$0 + 40{,}000\beta = -0.140.$

$\alpha = -0.000398.$

$\beta = -0.0000035.$

$X_m + \frac{\Sigma C_1^2}{n}\beta = D_m.$

X_m = density at mean temperature = $D_{35°}$.

D_m = mean of densities.

n = number of observations = 4.

$X_m = D_{35°} = D_m - 125B.$

$D_{35°} = 1.02995 + 0.00004_4 = 1.03039.$

$D_t = D_{35°} + \alpha(t-35) + \beta(t-35)^2.$

$D_t = 1.03039 - 0.000398(t-35) - 0.0000035(t-35)^2.$

Calculation for Dt

0.025 per cent butter fat (skim milk).

t	C_1	C_1^2	C_2	C_1C_2	C_2^2	D_t	N	C_1N	C_2N
°C.									
20	−15	225	+100	−1,500	10,000	1.0356	+0.00565	−0.08475	+0.565
30	− 5	25	−100	+ 500	10,000	1.0322	+ .00225	− .01125	− .225
40	+ 5	25	−100	− 500	10,000	1.0284	− .00155	− .00775	+ .155
50	+15	225	+100	+1,500	10,000	1.0236	− .00635	− .09525	− .635
35		4)500		0.000	40,000	1.02995		− .19900	− .140
		125							

0.025 per cent butter fat (skim milk).

t	$t-35$	$(t-35)^2$	$\alpha(t-35)$	$\beta(t-35)^2$	D_t (observed)	D_t (calculated)	Obs.−cal.
20	−15	225	+0.00597	−0.00079	1.0356	1.0356	0
30	− 5	25	+ .00189	− .00009	1.0322	1.0322	0
40	+ 5	25	− .00189	− .00009	1.0284	1.0284	0
50	+15	225	− .00597	− .00079	1.0236	1.0236	0

TABLE II.—*Densities of milk and cream corresponding to various percentages of fat*

Percentage of fat.	$D_{\frac{15}{15}}^{15°}$	$D_{\frac{20}{4}}^{20°}$	$D_{\frac{24}{4}}^{24°}$	α	β
	Sp. gr.	*Sp. gr.*	*Sp. gr.*		
0.025	1.037	1.035	1.030	−0.000040	−0.000005
1	1.036	1.034	1.029	.00040	.000004
2	1.035	1.033	1.028	.00040	.000004
3	1.034	1.032	1.027	.00041	.000004
4	1.032	1.031	1.025	.00041	.000003
5	1.031	1.029	1.024	.00041	.000003
6	1.030	1.028	1.022	.00042	.000003
7	1.029	1.027	1.021	.00042	.000002
8	1.027	1.026	1.020	.00043	.000002
9	1.026	1.024	1.018	.00044	.000001
10	1.025	1.023	1.016	.00045	.000001
11	1.024	1.022	1.015	.00046	.000000
12	1.022	1.020	1.013	.00047	.000000
13	1.020	1.019	1.012	.00048	.000000
14	1.019	1.017	1.010	.00048	+.000001
15	1.018	1.016	1.009	.00049	.000001
16	1.017	1.015	1.007	.00050	.000001
17	1.016	1.014	1.006	.00051	.000002
18	1.015	1.013	1.005	.00052	.000002
19	1.014	1.012	1.004	.00052	.000002
20	1.013	1.011	1.003	.00053	.000003
21	1.012	1.010	1.001	.00054	.000003
22	1.011	1.009	1.000	.00055	.000003
23	1.010	1.008	.999	.00057	.000004
24	1.009	1.007	.998	.00058	.000004
25	1.008	1.007	.997	.00059	.000004
26	1.008	1.006	.996	.00061	.000005
27	1.007	1.005	.994	.00062	.000005
28	1.006	1.004	.993	.00063	.000005
29	1.005	1.003	.992	.00064	.000005
30	1.004	1.002	.991	.00065	.000006
31	1.003	1.001	.990	.00066	.000006
32	1.002	1.000	.989	.00067	.000007
33	1.001	.999	.988	.00068	.000007
34	1.000	.998	.986	.00069	.000008
35	.999	.998	.985	.00071	.000008
36	.999	.997	.984	.00072	.000008
37	.998	.996	.983	.00073	.000009
38	.997	.995	.982	.00074	.000009
39	.996	.994	.981	.00075	.000009
40	.995	.993	.980	.00076	.000010

TABLE III.—*Sample set of observations—cream containing 25 per cent of fat*

[Samples received Nov. 19, 1913; observations made Nov. 20, 1913]

Temperature.				Balance readings.	Apparent weight.	Air buoyancy [a]	Corrected weight.	Weight of sinker in vacuo.	Displaced liquid weight.	Volume.	Density of liquid at observed temperature.	Correction to reduce to integral temperature.	Density of liquid reduced to integral degrees.	Integral temperature.
Observed		Corrected												
°C.	°C	°C.	°C.	*Gm*	*Gm.*	*Gm*	*Gm*	*Gm.*	*Gm.*	*C. c.*				°C.
[b] No. 2040:	[b] No. 4653													
	[c] + 0.04					0.00119								
20.50	20.48	20.50	20.52	31.7734	51.7631									
20.56	20.50	20.56	20.54	83.5365	51.7640									
Average		20.53		31.7725	51.7636	−.0073	51.7563	99.9630	48.2067	47.7135	1.01034	+0.00040	1.01074	20
0	0													
30.20	30.22	30.20	30.22	31.3950	52.1200									
30.20	30.21	30.20	30.21	83.5150	52.1215									
Average		30.21		31.3935	52.1208	−.0073	52.1135	99.9630	47.8495	47.7242	1.00262	+ .00014	1.00276	30
[b] No. 264:														
[c] −0.03	[c] +0.07													
39.84	39.80	39.81	39.87	31.1096	52.4012									
39.86	39.78	39.83	39.85	83.5108	52.3962									
Average		39.84		31.1146	52.3987	−.0074	52.3913	99.9630	47.5717	47.7350	.99658	− .00009	.99649	40
[b] No. 2036:	[b] No. 8998:													
[c] −0.03	[c] −0.30													
50.04	50.32	50.01	50.02	30.8460	52.6540									
50.12	50.38	50.09	50.08	83.5000	52.6480									
Average		50.05		30.8520	52.6510	−.0074	52.6436	99.9630	47.3194	47.7467	.99105	+ .00003	.99108	50

[a] In the column headed "Air buoyancy," 0.00119 is the air density and 0.0073 gm. is the buoyancy correction to be applied to the apparent weight.
[b] Number of the thermometer used
[c] Corrections for temperature.

TABLE IV.—*Observed and calculated densities of milk and cream at different temperatures and with different percentages of fat*[a]

Fat content.	D_{20}°	α	β	Temperature.	D_t. Observed.	D_t. Calculated.	Observed minus calculated.
Per cent.							
0.025	1.03039	−0.00040	−0.000004	20	1.0356	1.0356	0
				30	1.0322	1.0322	0
				40	1.0284	1.0284	0
				50	1.0236	1.0236	0
2.5	1.0276	−.00035	−.000005	20	1.0317	1.0317	0
				30	1.0292	1.0292	0
				40	1.0258	1.0258	0
				50	1.0212	1.0212	0
3.5	1.0259	−.00041	−.000003	20	1.0314	1.0314	0
				30	1.0279	1.0279	0
				40	1.0238	1.0238	0
				50	1.0191	1.0191	0
5	1.0259	−.00053	−.000006	20	1.0322	1.0327	[b]−5
				30	1.0299	1.0285	+14
				40	1.0217	1.0231	−14
				50	1.0171	1.0166	+5
5	1.0214	−.00042	−.000004	20	1.0268	1.0268	0
				30	1.0235	1.0234	+1
				40	1.0192	1.0193	−1
				50	1.0144	1.0144	0
7.5	1.0229	−.00038	−.000005	20	1.0274	1.0274	0
				30	1.0246	1.0247	−1
				40	1.0209	1.0208	+1
				50	1.0159	1.0159	0
7.5	1.0206	−.00042	−.000003	20	1.0261	1.0261	0
				30	1.0226	1.0226	0
				40	1.0184	1.0184	0
				50	1.0136	1.0136	0
10	1.0174	−.00045	0	20	1.0242	1.0242	0
				30	1.0197	1.0197	0
				40	1.0152	1.0152	0
				50	1.0106	1.0106	0
15	1.0086	−.00048	+.000006	20	1.0170	1.0171	−1
				30	1.0114	1.0111	+3
				40	1.0061	1.0063	−2
				50	1.0028	1.0027	+1
15	1.0086	−.00050	0	20	1.0161	1.0161	0
				30	1.0112	1.0111	+1
				40	1.0061	1.0061	0
				50	1.0011	1.0011	0
20	1.0023	−.00052	+.000002	20	1.0106	1.0107	−1
				30	1.0052	1.0050	+2
				40	.9995	.9997	−2
				50	.9950	.9949	+1

[a] The temperatures from which the reductions are made are the same for all samples—namely, 20°, 30°, 40°, and 50° C.—and for that reason C_1, C_2, etc., will be the same in all cases.

[b] The lack of agreement between the observed and the calculated values of density indicates that one or more of the observed values are considerably in error. All determinations on this sample should be discarded.

TABLE IV.—*Observed and calculated densities of milk and cream at different temperatures and with different percentages of fat*—Continued

Fat content.	D_{20}°	α	β	Temperature.	Dt.		Observed minus calculated.
					Observed.	Calculated.	
Per cent.							
25.....	0.9975	−0.00060	+0.000002	20	1.0070	1.0070	0
				30	1.0007	1.0006	+1
				40	0.9944	0.9945	−1
				50	0.9890	0.9890	0
25.....	.9995	−.00065	+.000000	20	1.0107	1.0107	0
				30	1.0028	1.0029	−1
				40	0.9965	0.9964	+1
				50	0.9911	0.9911	0
30.....	.9912	−.00057	0	20	0.9997	0.9997	0
				30	0.9940	0.9940	0
				40	0.9884	0.9884	0
				50	0.9827	0.9827	0
30.....	.9891	−.00051	−.000001	20	0.9966	0.9966	0
				30	0.9918	0.9917	+1
				40	0.9854	0.9865	−1
				50	0.9813	0.9812	+1
30.....	.9910	−.00049	−.000002	20	0.9978	0.9978	0
				30	0.9933	0.9934	−1
				40	0.9886	0.9885	+1
				50	0.9832	0.9832	0
30.....	.9920	−.00067	0	20	1.0044	1.0044	0
				30	0.9960	0.9962	−2
				40	0.9896	0.9894	+2
				50	0.9842	0.9842	0
35.....	.9841	−.00073	−.000008	20	0.9970	0.9970	0
				30	0.9879	0.9880	−1
				40	0.9807	0.9806	+1
				50	0.9750	0.9750	0
40.....	.9799	−.00074	−.000010	20	0.9931	0.9931	0
				30	0.9837	0.9838	−1
				40	0.9765	0.9764	+1
				50	0.9710	0.9710	0

These results were then plotted on coordinate paper of such a size that densities could be plotted and read to one in the fourth place, and α and β to one in the fifth and sixth places, respectively. From these curves the values of α and β for various densities of milk and cream are tabulated in Table II. The curves are shown on a reduced scale in figure 1.

In figure 2 is shown the relation between the density of the samples and the percentage of butter fat contained in them.

Having determined the density of each sample and the rate of change of density with change of temperature, it was possible to calculate the volume of any sample at any temperature in terms of the volume at any other temperature within the limits covered.

It was suggested by the Dairy Division of the Bureau of Animal Industry that 20° C. (68° F.) be chosen as the standard temperature and

that the relative volumes at other temperatures be given on the basis of unit volume at this temperature. It was also suggested that for the

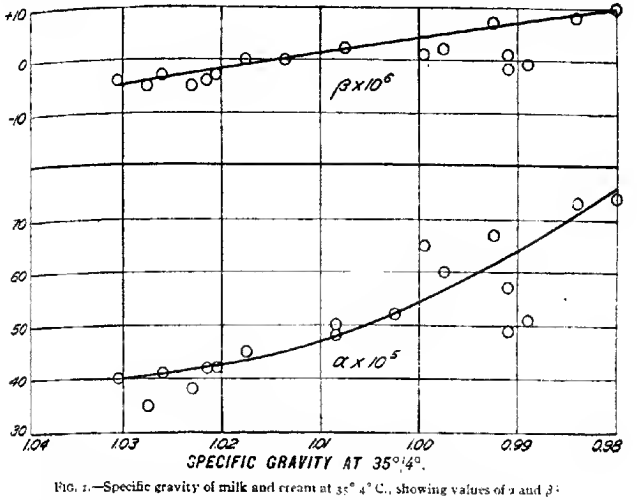

FIG. 1.—Specific gravity of milk and cream at 35° 4° C., showing values of α and β.

convenience of those by whom the table would be used the temperatures be given on the Fahrenheit scale, and that the densities at 20° C. in grams per cubic centimeter be changed to specific gravities at 20° C. in terms of

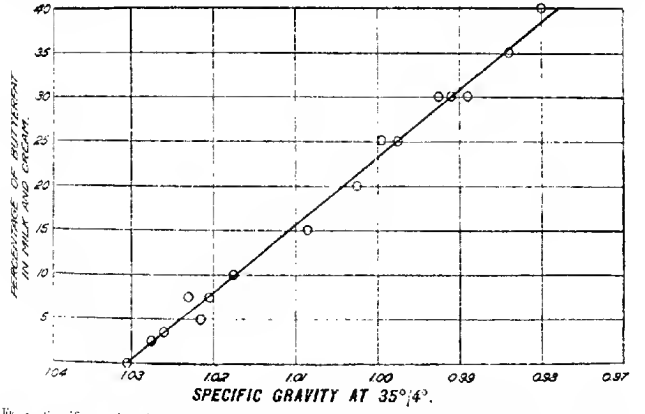

FIG. 2.—Specific gravity of milk and cream at 35°/4° C., showing relation between density and percentage of butter fat.

water at that temperature as unity. These changes have accordingly been made.

[1] The specific gravity at 35°/4° C. means the specific gravity at 35° C. in terms of water at 4° C. as unity. This is numerically the same as the density at 35° C. in gms. per c. c.

It was found that the calculation of volumes could be most conveniently accomplished by changing the basis of the calculation from 35° to 20° C. The equation

$$D_t = D_{35}° + \alpha(t-35) + \beta(t-35)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

was accordingly transformed to

$$D_t = D_{20}° + \alpha^1(t-20) + \beta^1(t-20)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

and the values of α^1 and β^1 determined. It can be shown that $\alpha^1 = \alpha - 30\beta$ and that $\beta^1 = \beta$. As a further convenience in calculation, the equation was changed to the form

$$V_t = V_{20}° \left[1 + A(t-20) + B(t-20)^2\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 3$$

in which A and B may be found in terms of α^1 and β^1.

It would, of course, be possible to calculate the volumes directly by means of equation 2, since $d = \frac{m}{V}$ or $V = \frac{m}{d}$, but the calculation is much more easily done by means of equation 3.

The volumes thus calculated are given in Table V.[1]

SOURCES OF ERROR

It has already been pointed out that one source of error is the gradual separation of the sample under investigation into its constituent parts, the fat rising to the top and the heavier portions settling toward the bottom. The greatest source of error, however, is probably in the assumed percentage of fat in the sample at the time the density determinations are made. This will be explained somewhat in detail. The samples of milk and cream were generally prepared at the Bureau of Animal Industry in the morning and brought to the Bureau of Standards in the afternoon of the same day. The density determinations were made on the following day. During this interval of time between the preparation of the samples and the making of the density determinations the samples could be kept sweet without difficulty, but there was in many cases a considerable amount of separation and "churning" of the fat, so that granules of butter were collected on the neck and the cap of the bottle in which the sample had been kept.

This change in the percentage of fat contained in the sample was, of course, always in such a direction that the sample at the time its density was determined contained a lower percentage of fat than that reported by the Bureau of Animal Industry at the time the sample was prepared. For that reason, since the density increases with decreasing percentage of fat, the densities of the different samples will in most cases be somewhat too large for the percentages of fat to which they are intended to correspond; or, in other words, the tabulated percentage of fat in a given sample is somewhat too high. In the tabulated values of percentage of fat and corresponding density (Table II), the density

[1] These data also appear as Table II, U. S. Dept. Agr. Bul. 98, p. 6-9.

is in each case given as corresponding to the percentage of fat in the sample at the time it was prepared. In reality it corresponds, not to that percentage of fat, but to the percentage in the sample at the time the density was determined. No attempt will be made at the present time to estimate how great this discrepancy may be; in some cases it is quite appreciable.

Other sources of error are the temperature observations and the weighings of the sinker. The weighings were made to tenths of a milligram and were probably correct in most cases to about half a mg. Errors greater than 1 or 2 mg. would be unlikely to occur. The thermometers were graduated to tenths of a degree centigrade and were read to hundredths. The mean of the four readings taken at each temperature was probably correct within one or two hundredths of a degree. Errors of more than three hundredths would not be expected. If both of these maximum errors should occur in the same set of observations and both should be in the same direction, the resulting error in the density would be about six units in the fifth decimal place. Even such an error would not be serious in the present instance, as the density values are used in the table only to the fourth place. The density determinations are almost certainly accurate to that degree. In the calculation of the densities the results were carried to the fifth place, and they are seen to be concordant in most cases to somewhat better than one in the fourth place.

CONCLUSION

Examination of the results here presented (see Table III) shows that for the individual samples examined the density determinations may be depended upon to about one unit of the fourth decimal place. These values, however, when plotted (see figs. 1 and 2), present certain irregularities which are far too great to be accounted for by errors in the determinations. For example, four different samples were examined, each of which was supposed to contain 30 per cent of fat. The densities of the four samples at 35° C. were found to be in satisfactory agreement, and for each sample the agreement between the observed and calculated densities at other temperatures was such as to throw no suspicion upon the determinations; and yet the rate of expansion of the four samples was widely different. Only one out of the four fitted reasonably well into the series formed by the samples above and below 30 per cent. This and similar anomalies for certain other samples make it appear that the rate of expansion of any given sample depends upon something more than the density or the percentage of fat present. It undoubtedly depends upon the physical and chemical condition of the sample at the time the observations are made. This condition is probably largely dependent upon the time that has elapsed since the preparation of the sample and upon the temperature at which it has been kept. That being the case, it would probably be impossible to find any fixed relation that would express accurately the rate of expansion of all percentages of butter fat under all conditions. Further investigation to determine the effect of time and temperature upon the rate of expansion would be of considerable interest, and such an investigation of these and similar points will be necessary before the rate of expansion under all ordinary conditions can be accurately known.

Although these results can not be considered as final, it is believed that for the purpose for which this work was undertaken results have been obtained which are of sufficient accuracy. It is, however, desirable that further work be done by a method better adapted to the nature of the liquid investigated. In future work greater precaution should be taken to prevent the fat from being removed from the samples before the density determinations are made, and it would be very desirable if the percentage of fat in each sample could be redetermined after the density determinations have been made. As these results were obtained with mixed milk it would also be desirable to compare the density of milk in different breeds of cows and the variation within the breeds.

TABLE V.—*Volume[a] of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*

Percentage of butterfat.	Temperature (° F.).											
	50	52	54	56	58	60	62	64	66	68	70	72
	Volume.											
0.025	0.9980	0.9980	0.9985	0.9985	0.9990	0.9990	0.9990	0.9995	0.9995	1.0000	1.0000	1.0005
1	.9980	.9980	.9985	.9985	.9990	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
2	.9975	.9975	.9980	.9980	.9985	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
3	.9975	.9975	.9980	.9980	.9985	.9990	.9990	.9995	.9995	1.0000	1.0000	1.0005
4	.9975	.9975	.9980	.9980	.9985	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
5	.9975	.9975	.9980	.9980	.9985	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
6	.9970	.9975	.9975	.9980	.9980	.9985	.9990	.9995	.9995	1.0000	1.0000	1.0005
7	.9970	.9970	.9975	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0000	1.0010
8	.9970	.9970	.9975	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
9	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
10	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
11	.9965	.9965	.9970	.9975	.9980	.9985	.9985	.9990	.9995	1.0000	1.0005	1.0010
12	.9955	.9960	.9965	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
13	.9955	.9960	.9965	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
14	.9950	.9955	.9960	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
15	.9950	.9955	.9960	.9970	.9975	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
16	.9950	.9955	.9955	.9965	.9970	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
17	.9945	.9950	.9955	.9965	.9970	.9980	.9985	.9990	.9990	1.0000	1.0005	1.0010
18	.9940	.9945	.9950	.9960	.9970	.9980	.9980	.9985	.9990	1.0000	1.0005	1.0010
19	.9940	.9945	.9950	.9960	.9970	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
20	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
21	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0005	1.0010
22	.9930	.9940	.9945	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0010	1.0015
23	.9930	.9940	.9940	.9955	.9965	.9975	.9980	.9985	.9990	1.0000	1.0010	1.0015
24	.9925	.9930	.9940	.9950	.9960	.9975	.9975	.9985	.9990	1.0000	1.0010	1.0015
25	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
26	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
27	.9925	.9930	.9940	.9950	.9960	.9970	.9975	.9985	.9990	1.0000	1.0010	1.0015
28	.9915	.9925	.9935	.9945	.9955	.9965	.9975	.9980	.9990	1.0000	1.0010	1.0015
29	.9915	.9925	.9935	.9945	.9955	.9965	.9975	.9980	.9990	1.0000	1.0010	1.0015
30	.9915	.9925	.9935	.9945	.9955	.9965	.9970	.9980	.9990	1.0000	1.0010	1.0020
31	.9915	.9925	.9935	.9945	.9955	.9965	.9970	.9980	.9990	1.0000	1.0010	1.0020
32	.9910	.9920	.9930	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
33	.9910	.9920	.9930	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
34	.9910	.9915	.9925	.9940	.9950	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
35	.9900	.9915	.9925	.9940	.9940	.9960	.9970	.9980	.9990	1.0000	1.0010	1.0020
36	.9900	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
37	.9890	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
38	.9890	.9910	.9920	.9930	.9940	.9955	.9965	.9980	.9990	1.0000	1.0010	1.0025
39	.9890	.9900	.9915	.9925	.9940	.9955	.9965	.9975	.9990	1.0000	1.0010	1.0025
40	.9890	.9900	.9915	.9925	.9940	.9950	.9960	.9975	.9990	1.0000	1.0010	1.0025

[a] The tabulated values are given to the nearest 0.0005.

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butter fat.	Temperature (° F.).											
	74	76	78	80	82	84	86	88	90	92	94	96
	Volume.											
0.025	1.0005	1.0010	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050
1	1.0005	1.0010	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050
2	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0040	1.0045	1.0050
3	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0045	1.0055
4	1.0010	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055
5	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0035	1.0045	1.0045	1.0050	1.0055
6	1.0010	1.0015	1.0020	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0050	1.0060
7	1.0010	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060
8	1.0010	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060
9	1.0010	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065
10	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065
11	1.0015	1.0020	1.0025	1.0025	1.0030	1.0035	1.0040	1.0045	1.0055	1.0055	1.0065	1.0070
12	1.0015	1.0020	1.0025	1.0030	1.0030	1.0035	1.0040	1.0050	1.0055	1.0060	1.0065	1.0070
13	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0065	1.0070
14	1.0015	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0070	1.0075
15	1.0015	1.0025	1.0030	1.0030	1.0035	1.0040	1.0045	1.0055	1.0060	1.0065	1.0070	1.0075
16	1.0015	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0055	1.0060	1.0070	1.0075	1.0080
17	1.0015	1.0025	1.0030	1.0035	1.0040	1.0045	1.0050	1.0060	1.0060	1.0070	1.0075	1.0080
18	1.0020	1.0025	1.0030	1.0035	1.0040	1.0045	1.0055	1.0060	1.0065	1.0075	1.0080	1.0085
19	1.0020	1.0025	1.0030	1.0035	1.0045	1.0045	1.0055	1.0060	1.0065	1.0075	1.0080	1.0085
20	1.0020	1.0025	1.0030	1.0035	1.0045	1.0050	1.0055	1.0060	1.0070	1.0075	1.0085	1.0090
21	1.0020	1.0025	1.0030	1.0040	1.0045	1.0050	1.0060	1.0065	1.0070	1.0080	1.0085	1.0090
22	1.0020	1.0030	1.0035	1.0040	1.0050	1.0055	1.0060	1.0065	1.0075	1.0080	1.0090	1.0095
23	1.0020	1.0030	1.0035	1.0040	1.0050	1.0055	1.0065	1.0070	1.0075	1.0085	1.0090	1.0095
24	1.0020	1.0030	1.0035	1.0040	1.0050	1.0060	1.0065	1.0070	1.0080	1.0080	1.0095	1.0100
25	1.0020	1.0030	1.0035	1.0045	1.0055	1.0060	1.0070	1.0075	1.0080	1.0090	1.0095	1.0105
26	1.0025	1.0030	1.0040	1.0045	1.0055	1.0060	1.0070	1.0080	1.0085	1.0090	1.0100	1.0110
27	1.0025	1.0030	1.0040	1.0045	1.0055	1.0060	1.0070	1.0080	1.0085	1.0095	1.0100	1.0110
28	1.0025	1.0030	1.0040	1.0045	1.0055	1.0065	1.0075	1.0080	1.0090	1.0095	1.0105	1.0115
29	1.0025	1.0030	1.0040	1.0050	1.0060	1.0065	1.0075	1.0080	1.0090	1.0095	1.0105	1.0115
30	1.0025	1.0035	1.0045	1.0050	1.0060	1.0065	1.0080	1.0085	1.0095	1.0100	1.0110	1.0120
31	1.0025	1.0035	1.0045	1.0050	1.0060	1.0065	1.0080	1.0085	1.0095	1.0100	1.0110	1.0120
32	1.0030	1.0035	1.0045	1.0055	1.0065	1.0070	1.0085	1.0090	1.0100	1.0105	1.0115	1.0125
33	1.0030	1.0035	1.0045	1.0055	1.0065	1.0070	1.0085	1.0090	1.0100	1.0105	1.0115	1.0125
34	1.0030	1.0040	1.0050	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0110	1.0120	1.0130
35	1.0030	1.0040	1.0050	1.0060	1.0070	1.0075	1.0090	1.0095	1.0105	1.0110	1.0120	1.0130
36	1.0035	1.0045	1.0055	1.0060	1.0070	1.0080	1.0090	1.0100	1.0110	1.0115	1.0125	1.0135
37	1.0035	1.0045	1.0055	1.0060	1.0070	1.0080	1.0095	1.0100	1.0110	1.0115	1.0125	1.0135
38	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0100	1.0115	1.0120	1.0130	1.0140
39	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0115	1.0120	1.0130	1.0140
40	1.0035	1.0045	1.0055	1.0065	1.0075	1.0085	1.0095	1.0105	1.0115	1.0125	1.0130	1.0145

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butter fat	Temperature (° F.)										
	98	100	102	104	106	108	110	112	114	116	118
	Volume.										
0.25	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105
1	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105
2	1.0055	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
3	1.0060	1.0065	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
4	1.0060	1.0065	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0110
5	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0085	1.0090	1.0095	1.0100	1.0110
6	1.0060	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0090	1.0095	1.0100	1.0110
7	1.0065	1.0070	1.0075	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0115
8	1.0065	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
9	1.0065	1.0070	1.0080	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
10	1.0070	1.0075	1.0080	1.0085	1.0090	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115
11	1.0070	1.0075	1.0080	1.0085	1.0090	1.0095	1.0095	1.0100	1.0105	1.0110	1.0115
12	1.0075	1.0080	1.0085	1.0090	1.0095	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125
13	1.0075	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125
14	1.0080	1.0085	1.0090	1.0095	1.0100	1.0100	1.0110	1.0115	1.0120	1.0125	1.0130
15	1.0080	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125	1.0130
16	1.0085	1.0090	1.0095	1.0100	1.0105	1.0110	1.0115	1.0120	1.0125	1.0130	1.0135
17	1.0085	1.0090	1.0095	1.0105	1.0105	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140
18	1.0090	1.0095	1.0100	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145
19	1.0090	1.0095	1.0100	1.0110	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145
20	1.0095	1.0100	1.0105	1.0110	1.0115	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150
21	1.0095	1.0100	1.0105	1.0115	1.0120	1.0125	1.0130	1.0135	1.0145	1.0150	1.0155
22	1.0100	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0150	1.0155	1.0160
23	1.0105	1.0105	1.0115	1.0120	1.0125	1.0130	1.0140	1.0145	1.0150	1.0155	1.0160
24	1.0105	1.0110	1.0120	1.0125	1.0130	1.0135	1.0145	1.0150	1.0155	1.0160	1.0165
25	1.0110	1.0115	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0160	1.0165	1.0170
26	1.0115	1.0120	1.0125	1.0135	1.0140	1.0145	1.0155	1.0160	1.0165	1.0170	1.0180
27	1.0115	1.0120	1.0130	1.0140	1.0140	1.0150	1.0155	1.0160	1.0170	1.0170	1.0180
28	1.0120	1.0125	1.0130	1.0140	1.0145	1.0150	1.0160	1.0165	1.0173	1.0175	1.0185
29	1.0120	1.0130	1.0135	1.0140	1.0150	1.0155	1.0160	1.0165	1.0175	1.0180	1.0185
30	1.0125	1.0130	1.0135	1.0145	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0190
31	1.0125	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175	1.0180	1.0185	1.0190
32	1.0130	1.0135	1.0140	1.0150	1.0160	1.0165	1.0170	1.0180	1.0185	1.0190	1.0195
33	1.0130	1.0140	1.0145	1.0155	1.0160	1.0165	1.0170	1.0180	1.0185	1.0190	1.0195
34	1.0135	1.0140	1.0150	1.0160	1.0165	1.0170	1.0175	1.0185	1.0190	1.0196	1.0200
35	1.0135	1.0145	1.0150	1.0160	1.0165	1.0170	1.0180	1.0190	1.0195	1.0200	1.0205
36	1.0140	1.0145	1.0155	1.0165	1.0170	1.0175	1.0185	1.0195	1.0200	1.0205	1.0210
37	1.0145	1.0150	1.0160	1.0165	1.0175	1.0180	1.0185	1.0196	1.0200	1.0205	1.0210
38	1.0150	1.0155	1.0165	1.0170	1.0175	1.0185	1.0190	1.0200	1.0210	1.0213	1.0218
39	1.0150	1.0160	1.0165	1.0170	1.0180	1.0185	1.0195	1.0205	1.0210	1.0215	1.0220
40	1.0155	1.0165	1.0170	1.0175	1.0185	1.0190	1.0200	1.0210	1.0215	1.0220	1.0230

TABLE V.—*Volume of milk and cream at various temperatures occupied by unit volume at 68° F. (20° C.)*—Continued

Percentage of butter fat.	Temperature (° F.).										
	120	122	124	126	128	130	132	134	136	138	140
	Volume.										
0.025	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
1	1.0110	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
2	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
3	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
4	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
5	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0155	1.0160	1.0170	1.0175
6	1.0115	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0153	1.0160	1.0170	1.0175
7	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0170	1.0175
8	1.0120	1.0125	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0165	1.0170	1.0175
9	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0180
10	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0153	1.0160	1.0165	1.0170	1.0180
11	1.0120	1.0130	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0180
12	1.0130	1.0135	1.0140	1.0145	1.0150	1.0153	1.0160	1.0165	1.0170	1.0175	1.0180
13	1.0130	1.0135	1.0140	1.0145	1.0150	1.0153	1.0160	1.0163	1.0170	1.0175	1.0180
14	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185
15	1.0135	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185
16	1.0140	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190
17	1.0145	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0175	1.0180	1.0185	1.0190
18	1.0150	1.0155	1.0160	1.0163	1.0170	1.0175	1.0180	1.0185	1.0185	1.0190	1.0195
19	1.0150	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0185	1.0190	1.0195
20	1.0155	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0195	1.0200	1.0205
21	1.0160	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0190	1.0195	1.0200	1.0205
22	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0190	1.0195	1.0200	1.0205	1.0210
23	1.0165	1.0170	1.0175	1.0180	1.0185	1.0190	1.0195	1.0193	1.0200	1.0205	1.0210
24	1.0170	1.0180	1.0185	1.0190	1.0193	1.0200	1.0205	1.0205	1.0210	1.0215	1.0220
25	1.0175	1.0180	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225
26	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235
27	1.0185	1.0190	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235
28	1.0190	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245
29	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245
30	1.0195	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0235	1.0240	1.0245	1.0250
31	1.0200	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0235	1.0240	1.0245	1.0250
32	1.0205	1.0210	1.0215	1.0220	1.0225	1.0230	1.0233	1.0240	1.0245	1.0250	1.0255
33	1.0205	1.0215	1.0215	1.0220	1.0225	1.0230	1.0233	1.0240	1.0245	1.0250	1.0255
34	1.0210	1.0215	1.0220	1.0225	1.0230	1.0240	1.0245	1.0245	1.0250	1.0255	1.0260
35	1.0210	1.0215	1.0220	1.0225	1.0230	1.0240	1.0245	1.0250	1.0250	1.0255	1.0260
36	1.0215	1.0225	1.0230	1.0233	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270
37	1.0215	1.0225	1.0230	1.0233	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270
38	1.0220	1.0230	1.0233	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270	1.0280
39	1.0225	1.0235	1.0240	1.0245	1.0250	1.0255	1.0260	1.0265	1.0270	1.0275	1.0280
40	1.0235	1.0240	1.0245	1.0275	1.0260	1.0265	1.0270	1.0275	1.0280	1.0285	1.0290

LIFE HISTORY OF THE MELON FLY

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INTRODUCTION

Aside from the Mediterranean fruit fly, *Ceratitis capitata* Wied., there is no other insect in the Hawaiian Islands that is causing such financial loss to fruit and vegetable interests as the melon fly, *Bactrocera cucurbitae* Coq. The damages caused by its ravages are placed by some even higher than those caused by *C. capitata*. While *B. cucurbitae* was not officially recorded until November, 1898, when it was first discovered by Mr. George Compere in the market gardens in the environs of Honolulu, it had been known locally about that city many years before. Mr. Albert Waterhouse, Acting President of the Hawaiian Board of Agriculture and Forestry, states that less than 30 years ago excellent cantaloupes (*Cucumis melo*) and watermelons (*Citrullus vulgaris*) and many kinds of pumpkins and squashes were grown in profusion the year round. Since that time the spread of the melon fly has been so rapid that this insect is now found on all the important islands of the Hawaiian group, and cantaloupes and watermelons can not be grown except on new land distant from old gardens. More than 95 per cent of the pumpkin (*Cucurbita pepo*) crop is annually ruined, not to mention the havoc caused among the more resistant cucumbers (*Cucumis sativus*).

Not only does the adult melon fly oviposit in fruit that has already set, but more often—in the case of the pumpkin and the squash (*Cucurbita* spp.)—in the unopened male and female flowers, in the stem of the vine, and even in the seedling itself, especially in seedlings of the watermelon and the cantaloupe. The writers have observed entire fields of watermelons killed before the plants were 6 to 8 inches long by larvæ boring into the taproot, stem, and leaf stalks. An examination of almost any pumpkin or squash field in the agricultural districts on the Island of Oahu at certain seasons of the year will show that very nearly all the flowers are affected before they have an opportunity to bloom. In about 95 cases out of 100 the anthers of the male bloom are either reduced to a mass of rot or more or less eaten before the bud becomes full grown, and the young ovaries of the female bloom are ruined by the burrowing maggots either before or shortly after the flower unfolds.

While cucurbitaceous crops are the favored host fruits of the melon fly, certain varieties of leguminous crops, such as string beans and cowpeas, are often badly attacked. When preferred host fruits are scarce, even peaches, papayas, and similar fruits are attacked to a limited degree. No satisfactory remedy has yet been found to prevent the infestation of fruit. The Chinese gardeners save a small percentage of crops subject to the attacks of this pest by covering the young fruit with cloth or paper or, in the case of the cucurbits, by burying them in the soil until they become sufficiently large to withstand attack.

The female melon fly deposits her eggs in small batches just beneath the surface of the fruit, vegetable, or plant affected. From these eggs

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 3
Dec. 15, 1914
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maggots are hatched which feed and burrow about, causing the rapid destruction of the affected parts, and then leave the host to enter the soil, where they pupate. After a short time the adult emerges from the pupa and soon deposits eggs for the following generation of larvæ.

THE EGG

From the data included in Table I it will be seen that the duration of the egg stage of the melon fly is very short. During the warm summer months, when the daily mean temperature is about 79° F., eggs hatch in 26 to 35 hours after deposition. The data indicate that hatching proceeds most rapidly about 27 or 28 hours after the eggs are laid. At a mean temperature of 75.6°, 84 eggs hatched in from 31 to 38 hours, while at a mean of 75°, 96 eggs hatched in about 47 hours after deposition. At 73.6°, 88 eggs hatched about 52 to 54 hours after being laid.

TABLE I.—*Duration of the egg stage of the melon fly*

Number of eggs under observation.	Eggs deposited		Eggs hatched		Average mean temperature for period
	Day.	Period.	Day.	Period	
					°F
17	Aug. 20	10.30 to 11.30 a. m.	Aug. 21	1 to 2 p. m.	79.5
75	do.	do.	do.	2 to 2.15 p. m.	79.5
45	do.	do.	do.	2.30 to 2.45 p. m.	79.5
12	do.	do.	do.	2.45 to 3 p. m.	79.5
23	do.	do.	do.	3 to 3.15 p. m.	79.5
9	do.	do.	do.	3.15 to 3.30 p. m.	79.5
6	do.	do.	do.	3.30 to 3.45 p. m.	79.5
20	do.	do.	do.	3.45 to 4 p. m.	79.5
9	do.	do.	do.	4 to 4.30 p. m.	79.5
37	do.	do.	do.	4.30 to 6 p. m.	79.5
7	do.	do.	do.	6 to 8 p. m.	79.5
5	do.	do.	do.	8 to 9 p. m.	79.5
2	do.	do.	do.	9 to 10 p. m.	79.5
84	May 19	3.15 to 3.30 p. m.	May 20	10 p. m. to 2 a. m.	75.6
96	May 14	4 to 6 p. m.	May 16	3 to 5.30 a. m.	75.0
13	May 13	10 a. m. to 1 p. m.	May 14	About 9 p. m.	75.5
62	do.	do.	do.	10 p. m. to 3 a. m.	75.5
77	May 11	11.30 a. m. to 2 p. m.	May 13	3 to 6 a. m.	73.6
11	do.	do.	do.	6 to 9.30 a. m.	73.6

THE LARVA

The larva of the melon fly passes through three instars before being full grown. The data in Table II show that at a mean temperature of about 79° F. larvæ can complete their development in from four days and four hours to seven days. The larvæ recorded as feeding upon papaya (*Carica papaya*) were transferred several times a day from one small piece of pulp to a fresh piece; hence, they probably pupated a few hours sooner than they would have pupated had they undergone their entire development in a single fruit. Larvæ developing in thick-skinned fruits, such as watermelons and pumpkins, often remain in the fruit after becoming full grown several days longer before emerging to pupate than they would have done had they been less confined. During the cooler seasons of the year the length of the larval life will probably be found to be much longer.

TABLE II.—*Duration of the larval stage of the melon fly*

Number of specimens under observation.	Approximate period of development.	Host fruit.	Number of hours in—			Period in larval stage.		Average mean temperature for period of development.
			Instar 1	Instar 2	Instar 3			
						Days.	*Hours*	°F.
1	Sept. 25 to 29.	Papaya	26	24	50	4	7	78.2
1	do	do	24	25	54	4	7	79
1	do	do	25	25	51	4	10	79
1	Sept. 25 to 30.	do	27	25	60	4	16	79
1	do	do	26	40	52	4	22	79
1	Sept. 29 to Oct. 4.	do	24	24	60	4	12	78.2
1	do	do	23	23.5	60	4	10	78.2
1	do	do	23.5	22	60	4	10	78.2
1	do	do	23.5	23.5	60	4	11	78.2
1	do	do	22.5	23	60	4	11	78.2
1	do	do	25.1	36	90	5	1	78.2
12	Aug. 22 to 26.	Cantaloupe				5		79
55	Aug. 22 to 27	do				6		79
28	Aug. 22 to 28.	Cucumber				6		79
42	Aug. 22 to 29.	Cantaloupe				7		79
36	do	Cucumber				7		79

THE PUPA

At mean temperatures varying from 71.6° to 79.4° F., the pupal stage ranges from 7½ to 13 days, as determined by observations on the 1,400 pupæ recorded in Table III. As the mean winter monthly temperatures seldom fall below 70° F., 13 days is probably close to the maximum length of the pupal stage during the cooler seasons in littoral Hawaii.

TABLE III.—*Duration of the pupal stage of the melon fly*[a]

Date of pupation.	Date of emergence.	Number of adults emerging.	Number of days of pupal stage.	Average mean temperature.
				°F.
Aug. 14, a. m.	Aug. 22, a. m.	5	8	78.7
Do.	Aug. 23, a. m.	25	9	78.9
Do.	Aug. 25, a. m.	1	11	78.9
Aug. 17, a. m.	do	60	8	79.3
Do.	Aug. 25, p. m.	1	8.5	79.3
Do.	Aug. 26, a. m.	27	9	79.2
Aug. 18, p. m.	do	2	7.5	79.3
Do.	Aug. 27, a. m.	10	8.5	79.4
Sept. 17, a. m.	Sept. 26, a. m.	26	9	79.2
Do.	Sept. 27, a. m.	37	10	79.2
Sept. 20, a. m.	Sept. 29, a. m.	355	9	79.4
Do.	Sept. 30, a. m.	33	10	79.3
Sept. 19, a. m.	Sept. 28, a. m.	300	9	79.4
Do.	Sept. 29, a. m.	15	10	79.3
Feb. 3, a. m.	Feb. 15, a. m.	45	12	71.6
Do.	Feb. 16, a. m.	192	13	71.6
Feb. 4, a. m.	do	38	12	71.6
Do.	Feb. 17, a. m.	228	13	71.6

[a] Total number of pupæ under observation: 1,400.

THE ADULT

The adults of the melon fly have proved most interesting from the standpoint of general hardiness, longevity, and oviposition.

LONGEVITY.—At the present time (Aug. 30, 1914) the writers have about 205 adults that emerged on February 17. They are, therefore, 6 months and 14 days old and are as strong and vigorous in appearance and action as when they emerged. Of the 248 adults alive on June 19, but 15 females and 28 males have died to date. If the death rate continues as low in the future, a few adults will probably live to be a year old.

SEXUAL MATURITY.—Neither male nor female melon flies are sexually mature when they emerge from the pupa. Out of about 200 individuals emerging on May 24, one pair was noted in coition on June 13, or 20 days after emergence. Among a second lot of adults, emerging on May 23, no adults mated until June 16, when two pairs were seen in coition. The majority of females in these lots did not mate until fully 25 days old. The daily mean temperatures for the period from May 23 to June 16 averaged 75.5° F. Sexual activity begins only at sunset. From sunset to dark copulation occurs and lasts in many instances until daybreak.

OVIPOSITION.—At mean temperatures averaging 75.5° F., females did not begin egg laying until about one month after eclosion. While fruit was placed in jars with about 1,000 adults which emerged from May 23 to 25, no attempts at oviposition were noted until June 23, when 12 punctures containing no eggs were made in a mango by females that emerged on May 24. The first eggs, 12 in number, were laid on June 25, or 32 days after eclosion. No eggs were obtained from females that emerged from May 25 until June 28, or 34 days after eclosion.

DAILY RATE OF OVIPOSITION.—While females do not begin ovipositing until about 1 month old, they continue to lay eggs for a long period. Thus, in Table IV is recorded the daily rate of oviposition of seven females during the first three months after emergence, while in Table V is recorded that of three females during the fourth, fifth, and sixth months of their life.

TABLE IV.—*Daily rate of oviposition of the melon flies that emerged on May 25 and were placed separately with fruit on June 25, 1914*

Date of observation.	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 10						23	
11			13		17		
12							
13							
14							
15	14					12	
16							
17			9		14		
18	19						
19							19
20							
21						6	
22	13						
23					10		

TABLE IV.—*Daily rate of oviposition of the melon flies that emerged on May 25 and were placed separately with fruit on June 25, 1914*—Continued

Date of observation.	Number of eggs deposited.						
	Fly No. 1.	Fly No. 2.	Fly No. 3.	Fly No. 4.	Fly No. 5.	Fly No. 6.	Fly No. 7.
July 24				3			
25							
26						23	
27	29						
28							
29					21		
30							
31							
Aug. 1				10		5	
2			15				
3							
4		2			15		
5				25			
6	16						
7		6					
8					10	8	
9	19				3		
10							
11				6		1	
12					2		
13					10		
14	16						23
15			10				
16						1	
17					5	2	9
18				11			
19	12						
20							
21							
22							
23						8	
24	17	12			9		
Total	155	20	47	55	116	89	51

TABLE V. *Daily rate of oviposition of melon flies that emerged on February 17 and were placed separately with fruit on May 22, 1914*

Date.	Number of eggs deposited.			Date.	Number of eggs deposited.		
	Fly No. 1.	Fly No. 2	Fly No. 3.		Fly No. 1.	Fly No. 2	Fly No. 3
May 22	21			June 1			
23				2			
24				3			
25				4			
26				5			
27				6		17	
28				7			
29				8		13	
30	14	2		9			
31	4		2	10			

to about 32 diameters exhibits very definite hairy characters, as described below.

Pammel and King (6, p. 170) published a brief account of the seeds of *A. repens* and *A. smithii* in which the main points of difference are pointed out as being found in the shape of the palea and in the hairs on its face and edge. While these distinctions are in the main correct, they are insufficient for a complete diagnosis. As to shape of the seeds, these authors make the following statement:

> The seed of quack grass is more slender and spindle-shaped, while that of western wheat grass broadens out somewhat toward the tip, after the manner of brome and some other grasses.

According to the results of the examinations of a large number of seeds, the writer finds that the difference in shape above noted is not constant. Hence, its use as a single determining character is not warranted.

In a taxonomic key of seeds of Agropyron issued by Sarvis (7, p. 2) the rachilla of *A. repens* is described as being "puberulent, each hair being glandular at the base." According to the writer's observations, the rachilla would more properly be described as hirsutulous. Moreover, in order to discern the glands at the base of the hairs, a compound microscope is required, which makes the use of this character impracticable for ordinary seed-laboratory methods. Even with such high power, the glands are not always clearly discernible. A glabrous palea in *A. repens* and a hispid palea in *A. smithii* are indicated by Sarvis as important characters in the determination of the Agropyron species. While this is true for the majority of seeds of these species, the writer has found many seeds in which this distinction does not hold. These characters intergrade to such an extent that they are not only unreliable, but are misleading as a single diagnostic criterion. Sarvis also holds that the tip of the palea of *A. tenerum* is very puberulent. This is true not only for the species above mentioned but also for *A. repens* and *A. smithii*.

SEED CHARACTERS OF SPECIES OF AGROPYRON

In the determination of seeds of Agropyron there are no absolutely fast and definite single characters by which a seed of one species may be unfailingly distinguished from the seed of any other species. Variation is found not only in the seed but also in the other unit parts of the plants, particularly in the spikes and spikelets (Pls. XXXIV, XXXV, and XXXVI). Moreover, seeds growing in different localities may exhibit considerable variation. This variation necessitates a close study of numerous characters of each seed, and any diagnosis to be of value must be based on a large number of seeds collected from a wide range and under widely differing conditions.

It is also obvious that the larger the number of seed characters which are studied the greater will be the possibility of making an accurate determination of the species under examination. A single character may vary to such an extent as to be quite untypical of the species, and consequently a determination based upon only one character may be incorrect, and therefore misleading. The necessity for an intimate knowledge of several distinguishing characters is even more

pronounced when Agropyron seeds become so mutilated that portions of the glumes are destroyed, as is frequently the case in commercial seed mixtures. In some cases the glumes are entirely gone, leaving only the grain, and determinations according to characters described below then become impossible. No satisfactory method of real practical value has yet been worked out whereby the seeds without the glumes may be accurately determined, and it seems probable that in such cases one may be compelled to resort to microscopic sections. One characteristic difference may be noted, however—namely, that the color of the matured grain of *A. tenerum* is somewhat lighter than that of either *A. repens* or *A. smithii*. The two latter approximate each other closely in color.

SOURCE OF SEEDS

Materials for this work were secured from as many sources as possible, as given in Table I. Only those samples have been considered which were obtained from and determined by a competent botanist, who was sure of the origin of the seed.

TABLE I.—*Sources of seeds of Agropyron spp. used in investigation*

Source of seed	A. repens.	A. smithii.	A. tenerum.
Canada (Manitoba)			(a)
Illinois (Chicago) [b]		(a)	(a)
Iowa	(a)	(a)	(a)
Michigan	(a)		
Minnesota	(a)	(a)	(a)
New York (Geneva)	(a)		(a)
North Dakota	(a)	(a)	(a)
North Dakota [b]	(a)		(a)
Russia [b]	(a)		
Washington (State)	(a)	(a)	(a)
Wisconsin [b]		(a)	(a)
Wisconsin	(a)		(a)
Wyoming		(a)	(a)

[a] Sample received.
[b] From Seed Laboratory of United States Department of Agriculture, Washington, D. C.

LABORATORY METHODS OF IDENTIFICATION

It is obviously necessary that all methods of identification, especially for use by farmers or seedsmen and even for seed laboratories, be as simple as possible and that they do not require elaborate or expensive apparatus. If, however, the distinguishing characters are not visible to the naked eye or with the aid of an ordinary magnifying glass, then it becomes absolutely necessary to use a higher power of magnification.

In the identification of seeds of *Agropyron* spp. it is advisable to use a magnification of about 32 diameters for the best results. The Greenough binocular giving the stereoscopic view has proved very satisfactory. It is absolutely necessary when examining seeds under the lens to place them so that the base of the seed is toward the light, in which position the light will be properly reflected from the hairs, making them appear clear and well defined.

SHAPE OF SEED

The seed of *A. repens* (fig. 1, *A*) has its point of greatest divergence about midway between the base and the tip, differing in this respect from *A. tenerum* (fig. 1, *C*), which has its point of greatest divergence about one-third of the length of the seed from the tip. The lemma and the palea of the latter species flare out more or less at this point, thus making the seed look flattened and thin. In the majority of cases the seed is unsymmetrical in shape, the top portion of the glumes being affected by a lateral displacement, as shown in the illustration. This makes possible a quick and accurate determination of a bulk lot of seeds of *A. tenerum*. The seed of *A. smithii* (fig. 1, *B*) has the same general shape as that of *A. repens*, but it is larger and has a more robust appearance.

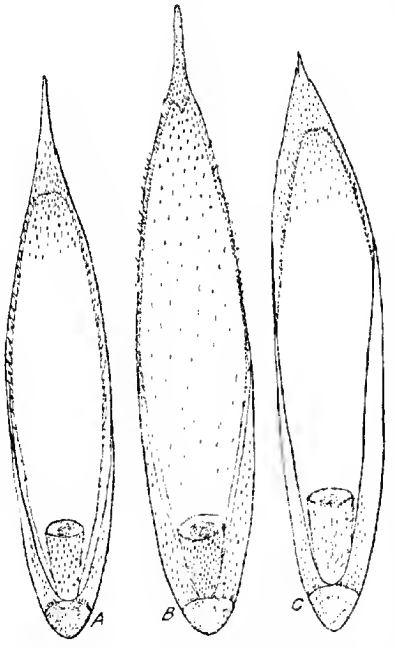

FIG. 1.—Detail drawings of dorsal view of *Agropyron* spp.: *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

RACHILLA

It is impossible to describe very definitely the characteristics of the rachilla of the different species of Agropyron because of the variation. In a general way the sides of the rachilla of *A. repens* are more nearly parallel, and the rachilla itself is more or less appressed to the palea. In *A. smithii* the sides of the rachilla diverge noticeably more from the point of its attachment. The rachilla stands out more prominently from the seed, being materially different in this respect from *A. repens* (fig. 2). The rachilla of *A. tenerum* has no particularly characteristic shape, varying from the slender to the short, stout, diverging type. A very good idea of relative size and shape of these seeds may also be gained by studying Plate XXXVII, which shows typical seeds, together with a typical spikelet of each of the three species.

The hairs clothing the rachilla constitute a valuable character used in the determination of the seed. However, care and good judgment must be exercised because of the great variation which may occur.

The characteristic rachilla of *A. repens* (fig. 1, *A*) is sparsely covered with short, minute hairs having a rather large base. Occasionally a glandular structure may be discerned at the base. This, however, can only be seen with a high-power lens and is not considered of sufficient

importance to warrant its use as a determining character. No rachilla of *A. repens* has been found which had the hirsute character of *A. smithii* (fig. 1, *B*) or the pilose character of *A. tenerum* (fig. 1, *C*).

The rachilla of *A. smithii* is characterized by hairs of the same general shape as the hairs found on the rachilla of *A. repens*. They are, however, larger and stronger and the number is noticeably greater. This characteristic is fairly uniform.

The rachilla of *A. tenerum* is characterized by hairs of a pilose nature. They are long as compared with those of *A. repens* and *A. smithii*, and may often be distinguished by this feature alone from these two species, as the pilose nature has never been observed on them. However, an absolutely authentic specimen of *A. tenerum* has been examined which had a rachilla much resembling that of *A. repens*. The hairs were short, but were not as large as the base. Other characters on the seed, however, made it possible to place it accurately in the species *tenerum*.

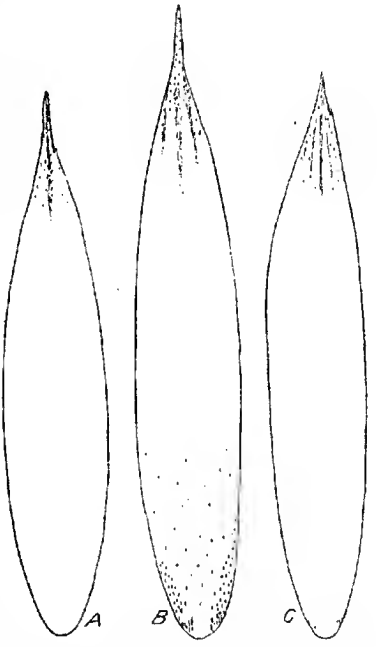

FIG. 2.—Side views of basal portions of seeds of *Agropyron* spp., showing the relative projection of the rachilla. *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

LEMMA

Another distinguishing character and one which is reliable as to uniformity may be found at the base of the lemma on the ventral side of the seed. In *A. tenerum* (figs. 2, *C*, and 3, *C*) there is a line of hairs which extends from the base of the rachilla on the dorsal side of the seed around and entirely across the face of the lemma on the ventral side near the base of the seed. In some cases it may be impossible to distinguish the hairs on the middle of the lemma, but the surface of the lemma at this point is roughened sufficiently so that it is noticeable. This is a fairly definite character.

The seed of *A. repens* (figs. 2, *A*, and 3, *A*) has no such characteristic line of hairs, but the basal portion of the lemma is entirely smooth and shiny. This character in the seed of *A. smithii* (fig. 3, *B*) is somewhat variable and is therefore not of much value. Most commonly, however, it is found that the ring of hairs extends part way around on either side, and on the middle of the lemma there is a space which usually is entirely smooth.

PALEA

The part of the seed which discloses good and reasonably definite characteristic differences is the palea. The face of the palea in *A. repens* and *A. tenerum* (figs. 1, *A*, and 1, *C*) is practically glabrous, except near the tip, where it is puberulent. Occasionally there is a small number of hairs distributed over the face of the palea. Since the tips of the paleæ in both of these species are always puberulent, this can not be used as a distinguishing character. The palea of the seed of *A. smithii* (fig. 1, *B*) is quite hirsute over its entire surface.

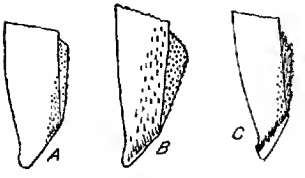

FIG. 3.—Detail drawings of ventral view of seeds of *Agropyron* spp.: *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

The hairs on the edge of the palea have a distinctive shape for each of the three species and are very useful as a determining factor. Those of *A. repens* (fig. 4, *A*) are rather short, stout, and somewhat blunt. Those of *A. smithii* (fig. 4, *B*) are about as coarse as those on *A. repens*, but are noticeably longer, thus making them appear more slender. On *A. tenerum* (fig. 4, *C*) the hairs are finer, closer together, and more acutely pointed than in the case of the two others.

The palea of *A. smithii* (fig. 1, *B*) has a very characteristic tip, which character runs fairly uniform throughout the species. The tip of the palea is definitely divided, making a well-defined cleft (Pl. XXXVII, 2, *a*). In some cases it is rather difficult to distinguish this cleft, as the lobes may be slightly overlapped. The tips of the palea of *A. repens* and *A. tenerum* are simply rounded or only slightly indented.

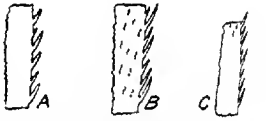

FIG. 4.—Edge of rachilla in *Agropyron* spp., showing shape and comparative size of bristles; *A*, *Agropyron repens*; *B*, *A. smithii*; *C*, *A. tenerum*. × 9.

SUMMARY

It is possible by careful examination to distinguish in commercial seed mixtures the seeds of the three species of Agropyron: *A. repens*, *A. smithii*, and *A. tenerum*.

There is no one character which can unfailingly be relied upon for this diagnosis, but the combined characters of lemma, palea, and rachilla are necessary for a safe determination.

Probably the nearest approach to a single critical structure is found in the palea, which exhibits fairly definite characters in each of the species.

The diagnostic differences are summarized in Table II.

TABLE II.—*Diagnostic differences of Agropyron spp.*

Character.	A. repens.	A. smithii.	A. tenerum.
Shape of seed	Boat-shaped	Boat-shaped	Widest one-third of distance from the tip, which is more or less flattened.
Rachilla	Sides approximately parallel. Hairs few, short, and stout.	Sides divergent. Hairs numerous, stout, but longer than those which characterize A. repens.	Variable in shape and size. Hairs numerous, slender, and long
Palea: Face	Puberulent at tip; otherwise glabrous.	Hirsute over entire face.	Puberulent at tip. Remaining surface glabrous.
Edges	Characterized by short, stout, and blunt hairs.	Hairs stout, but longer than those of A. repens.	Hairs fine, acute, and close together
Tip	Rounded or indented	Cleft	Rounded or indented.
Lemma	Smooth and shiny at base on ventral side.	Usually with a break in the line of hairs on ventral side at base of seed.	Line of hairs extends across lemma at its base.

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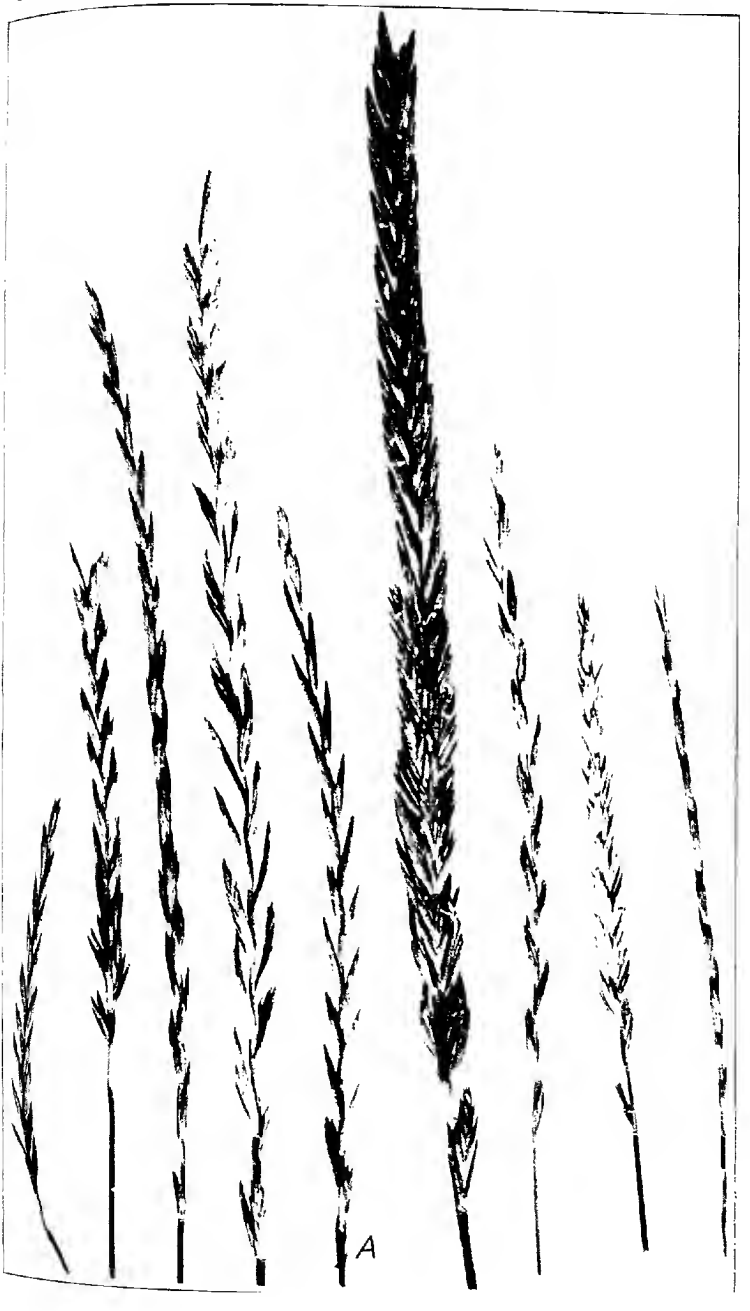
PLATE XXXIV

Agropyron repens: Spikes showing degrees of variation which may occur. A, typical spike. A side view of this spikelet may be seen at top portion of this spike. Natural size.


A

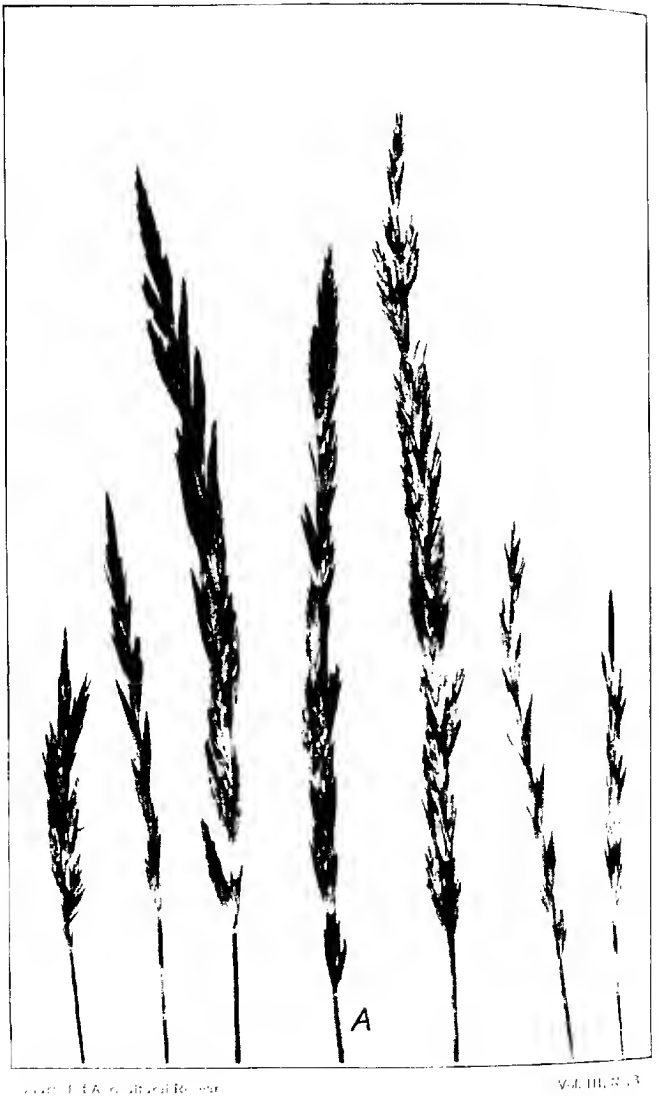
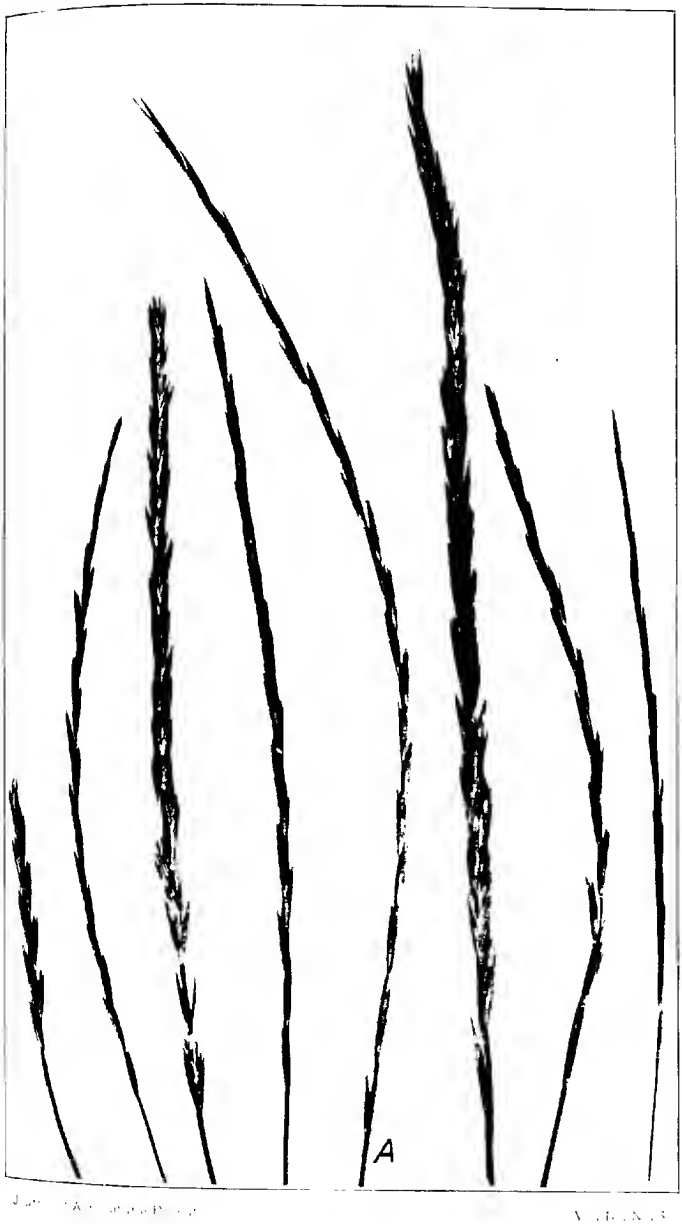

A

PLATE XXXV

Agropyron smithii: Spikes showing degrees of variation. A, typical spike. Natural size.

PLATE XXXVI

Agropyron tenerum: Spikes showing degrees of variation. A, typical spike. Natural size.


A

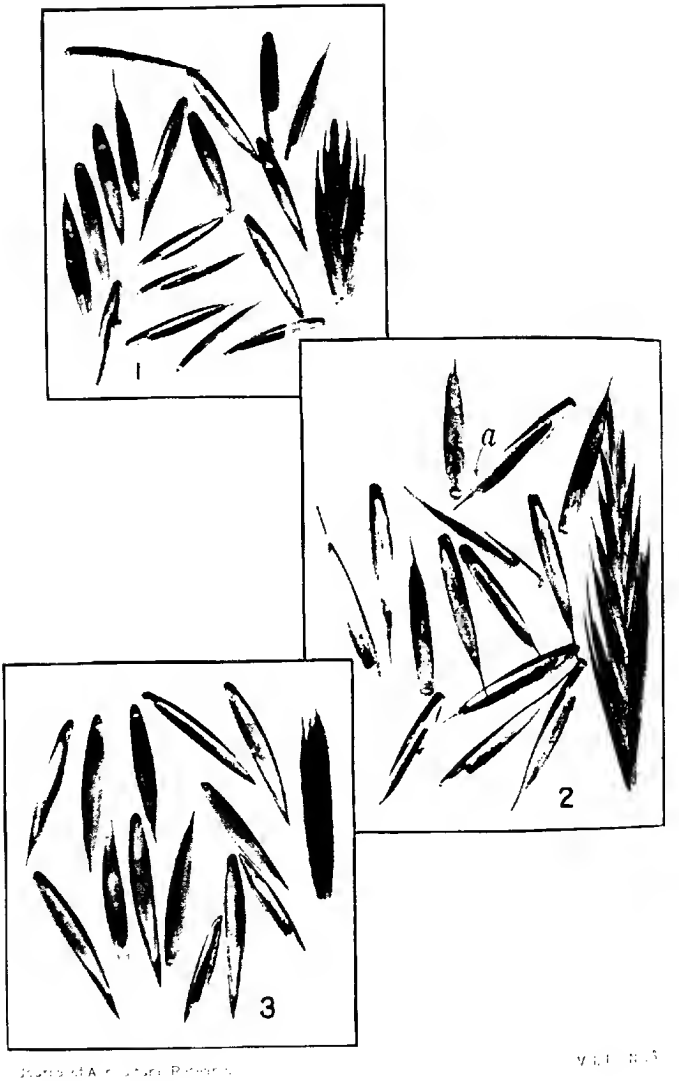

1
a
2
3

PLATE XXXVII

Agropyron spp.: Typical seeds and spikelets. Enlarged.

Fig. 1.—*Agropyron repens*.
Fig. 2.—*Agropyron smithii*.
Fig. 3.—*Agropyron tenerum*.